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# CERTAIN EVOLUTIONARY ASPECTS OF HUMAN MORTALITY RATES[1]

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## I

IT is the purpose of this paper to set forth some facts regarding human mortality which appear to lead with great clarity to certain evolutionary generalizations of interest to the biologist, which have hitherto been overlooked so far as I am aware. The present fashion in the study of evolution is towards the analytical discussion of the factors. Synthetic general discussions of broad phases of organic evolution, which occupied so prominent a place in early post-Darwinian times, are now but rarely found in biological literature. This may fairly be regarded as a blessing, but perhaps not an entirely unmitigated one. While much of the general discussion of evolution of the period of fifty years ago was utter nonsense, still a view of some of the aspects of the forest may be at least occasionally stimulating, and particularly in these present days when we are accumulating such a mass of precise data about the characteristics of the trees.

It is in some ways remarkable that so little thought and interest have been given by general biologists to the phases of biology which form the working material of that branch of applied science which is roughly but still sufficiently intelligibly labelled "vital statistics." The data

[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 7.

In preparing this paper I have had the benefit in matters of pathological anatomy and embryology of the critical acumen and wide knowledge of my colleague, Dr. W. T. Howard, to whom I am greatly indebted for this help.

of human natality, morbidity and mortality, when intelligently and broadly studied, can, I am sure, throw a great deal of light on some of the deepest and most significant problems of general biology. If the facts presented in this paper succeed in some small degree in demonstrating that this opinion is not an entirely idle one, the purpose of this particular piece of work will have been served.

## II

By an international agreement among statisticians the causes of human mortality are, for statistical purposes, rather rigidly defined and separated into something over 180 distinct causes. It should be clearly understood that this convention is distinctly and essentially statistical in its nature. In recording the statistics of death the vital statistician is confronted with the absolute necessity of putting every death record into some category or other in respect of its causation. However complex biologically may have been the train of events leading up to a particular demise, the statistician must record the terminal "cause of death" as some particular thing. The International Classification of the Causes of Death is a code which is the result of many years' experience and thought. Great as are its defects in certain particulars, it nevertheless has certain marked advantages, the most conspicuous of which is that by its use the vital statistics of different countries are put upon a uniform basis.

The several separate causes of death are grouped in the International Classification into the following general classes:

I. General diseases.
II. Diseases of the nervous system and of the organs of special sense.
III. Diseases of the circulatory system.
IV. Diseases of the respiratory system.
V. Diseases of the digestive system.
VI. Non-venereal diseases of the genito-urinary system and annexa.

VII. The puerperal state.
VIII. Diseases of the skin and of the cellular tissue.
IX. Diseases of the bones and of the organs of locomotion.
X. Malformations.
XI. Early infancy.
XII. Old age.
XIII. External causes.
XIV. Ill-defined diseases.

It is evident enough that this is not primarily a biological classification. The first group, for example, called "General diseases," which caused in 1916, in the Registration Area of the United States approximately one fourth of all the deaths, is a curious biological and clinical melange. It includes such diverse entities as measles, malaria, tetanus, tuberculosis, cancer, gonococcus infection, alcoholism, goiter, and many other equally unlike causes of death. For the purposes of the statistical registrar it has useful points to make this "General diseases" grouping, but it clearly corresponds to nothing natural in the biological world. Again, in such part of the scheme as does have some biological basis, the basis is different in different rubrics. Some of the rubrics have an organological base, while others, as "Malformations" have a causational rather than an organological base.

Altogether it is evident that if any synthetic biological use is to be made of mortality data a fundamentally different scheme of classification of the causes of death will have to be worked out.

III

For the purposes of this study[2] I have developed an

[2] It should be clearly understood that this phrase "For the purposes of this study" means precisely what it says. I am not advocating a new classification of the causes of death for statistical use. I should oppose vigorously any attempt to substitute a new classification (mine or any other) for the International List now in use. Uniformity in statistical classification is essential to usable, practical vital statistics. Such uniformity has now become well established through the International Classification. It would be most

entirely different general classification of the causes of death on a reasonably consistent biological basis. The underlying idea of this new classification is to group all causes of death under the heads of the several organ systems of the body, the functional breakdown of which is the immediate or predominant cause of the cessation of life. All except a few of the statistically recognized causes of death in the International Classification can be assigned places in such a biologically grouped list. It has a sound logical foundation in the fact that, biologically considered, death results because some organ system, or group of organ systems, fails to continue its function. Practically, the plan involves the reassignment of all of the several causes of death now grouped by vital statisticians under heading "I. General diseases." It also involves the re-distributing of causes of death now listed under the puerperal state, malformations, early infancy, and certain of those under external causes.

The headings finally decided upon for the new classification are as follows:

I. Circulatory system, blood, and blood-forming organs.
II. Respiratory system.
III. Primary and secondary sex organs.
IV. Kidneys and related excretory organs.
V. Skeletal and muscular systems.
VI. Alimentary tract and associated organs concerned in metabolism.
VII. Nervous system and sense organs.
VIII. Skin.
IX. Endocrinal system.
X. All other causes of death.

It should be emphasized before presenting the tables

undesirable to make any radical changes in the Classification now. I have in this paper made a rearrangement of the causes of death, for the purposes of a specific biological problem, and no other. I am not "proposing a new classification of vital statistics" for official or any other use except the one to which I here put it.

of detailed statistics on this new classification that the underlying idea of this rearrangement of the causes of death is to put all those lethal entities together which bring about death because of the functional organic breakdown of the same general organ system. The cause of this functional breakdown may be anything whatever in the range of pathology. It may be due to bacterial infection; it may be due to trophic disturbances; it may be due to mechanical disturbances which prevent the continuation of normal function; or to any other cause whatsoever. In other words, the basis of the present classification is not that of pathological causation, but it is rather that of organological breakdown. We are now looking at the question of death from the standpoint of the pure biologist, who concerns himself not with what causes a cessation of function, but rather with what part of the organism ceases to function, and therefore causes death. It is to be hoped that the novelty of this method of looking at the causes of human mortality will not *per se* prejudice the reader against it, to the degree at least of preventing him from examining the detailed results and consequences of such classification, which will be presented in what follows.

There will now be presented in a series of tables the statistical data as to deaths arranged in this classification. The data given are in the form of death rates per hundred thousand living at all ages from various causes of death, arranged by organ systems primarily concerned in death from the specified disease. The statistics presented are from three widely separated localities and times, viz., (*a*) from the Registration Area of the United States; (*b*) from England and Wales; and (*c*) from the City of Sao Paulo, Brazil. The first two columns of each table give the death rates, arranged in descending order of magnitude in the first column, for the Registration Area of the United States for the two periods, 1906–10 and 1901–05. The third column of each table gives the death rate from the same cause of death for England and

Wales in the year 1914. The fourth column gives the rates for Sao Paulo for the year 1917. The data for the United States Registration Area were extracted from the volume of Mortality Statistics for 1916, issued by the Bureau of the Census. The English data were extracted from the Report of the Registrar General of England and Wales for 1914. The Sao Paulo rates were calculated from data as to deaths and population given in the "Annuario Demographico" of Sao Paulo for 1917.

TABLE I

CIRCULATORY SYSTEM, BLOOD AND BLOOD-FORMING ORGANS

| No.[3] | "Cause of Death" as per International Classification | Registration Area, U.S.A. | | England and Wales 1914 | Sao Paulo 1917 |
|---|---|---|---|---|---|
| | | 1906–10 | 1901–05 | | |
| 79 | Organic diseases of the heart | 133.2 | 124.2 | 137.3 | 130.0 |
| 81 | Diseases of the arteries | 17.7 | 9.4 | 23.5 | 59.7 |
| 78 | Acute endocarditis | 12.2 | 11.2 | 5.1 | 6.5 |
| 7 | Scarlet fever | 10.6 | 11.0 | 7.7 | 5.4 |
| 150[4] | Congenital malformation of the heart | 9.0 | 6.7 | 4.2 | 4.6[5] |
| 80 | Angina pectoris | 6.8 | 6.6 | 3.2 | 2.2 |
| 82 | Embolism and thrombosis | 3.9 | 4.2 | 8.9 | 8.3 |
| 20 | Purulent infection and septicemia | 3.8 | 6.1 | 1.8 | 22.2 |
| 142 | Gangrene | 3.5 | 4.5 | 4.4 | 2.6 |
| 4 | Malaria | 2.6 | 4.8 | 0.2 | 2.8 |
| 85 | Hemorrhage and other diseases of the circulatory system | 1.6 | 2.8 | 0.6 | 2.4 |
| 53 | Leukemia | 1.5 | 1.2 | 2.0 | 2.0 |
| 77 | Pericarditis | 1.3 | 2.1 | 1.2 | 1.1 |
| 54 | Anemia, chlorosis | 1.0 | 0.5 | 6.4 | 3.7 |
| 83 | Diseases of the veins | 0.6 | 0.6 | 1.0 | 0.7 |
| 84 | Diseases of the lymphatics | 0.3 | 0.2 | 0.9 | 0.2 |
| 116 | Diseases of the spleen | 0.2 | 0.3 | 0.2 | 0.4 |
| 16 | Yellow fever | 0 | 0.3 | 0 | 0 |
| 15 | Plague | 0 | 0.1 | 0 | 0 |
| 2 | Typhus fever | [6] | [6] | 0 | 0 |
| 3 | Relapsing fever | [6] | [6] | 0 | 0 |
| 11 | Miliary fever | [6] | [6] | 0 | 0 |
| | Totals | 209.8 | 196.8 | 208.6 | 254.8 |

Nine of the items in Table I, namely items 77 to 85 inclusive, are those of rubric III of the International Class-

[3] The numbers in this column in this and the following tables are the numbers of the several causes of death in the International Classification.

[4] In part.

[5] The Sao Paulo statistics do not separate congenital malformations. This is the total rate.

[6] Less than 0.1 per 100,000.

ification, "Diseases of the circulatory system." The other items of Table I require some special explanation.

No. 7, "Scarlet fever," appears in the International Classification under "General diseases." It is placed here in the organological classification because in the vast majority of cases of fatal scarlet fever it is the clinical form of the disease known as septic scarlatina which is responsible for the death. Spengarn[7] says that "septic scarlatine is responsible for most of the deaths." "The general condition is one of septicemia." It, therefore, seems best, on the present plan of biological classification, to put scarlet fever with the circulatory system, blood and blood-forming organs, since septicemia is the result of a breakdown and failure to function of the normal defensive serologic mechanism of the body.

The item 150 in the International Classification is entitled "Congenital malformations," and there includes the following three subdivisions: Hydrocephalus, congenital malformations of the heart, and other congenital malformations. The second of these subdivisions, "congenital malformations of the heart," obviously belongs here, and is consequently included, while the other subdivisions do not.

Item 20, "Purulent infection and septicemia," is taken from "General Diseases" and put here on the same reasoning as that just stated for scarlet fever.

Item 142, "Gangrene," is placed here because normally in civilian life, under the conditions which prevailed when these statistics were taken, most fatal gangrene is due to impairment of the circulation as a primary cause. The arteries become occluded either from endarterial inflammation, due either to frank infection, or to various somewhat obscure causes producing local obliterative arteriosclerosis, or to trauma, or to thrombosis or embolism, especially in association with cardiac disease. Again some cases of gangrene, in the sense under con-

[7] Spengarn, A., article "Scarlatine," in Ref. Handbook Med. Sci., Vol. VII, p. 658, 1916.

sideration here, are doubtless due to extensive phlebitis and primary thrombosis of veins. In any case it is a part of the circulatory system which breaks down, and therefore we are warranted in placing this disease in Table I.

Item 4, "Malaria," is fundamentally a disease of the blood, and hence is placed here from "General diseases."

All the evidence that the pathological anatomist has leads to the view that yellow fever, typhus fever, relapsing fever and miliary fever are blood diseases. They have the lesions of septicemias, or are transmitted by biting insects, or both.

Items 53 and 54, "Leukemia" and "Anemia, chlorosis," represent breakdowns of the blood or blood-forming organs of the body. They are taken from Class I of the International Classification.

In the International Classification item 116, "Diseases of the spleen," is placed under the general rubric of "Diseases of the digestive system." This is a good illustration of the biological absurdities which appear in the statistical classification now used. Just what the spleen has to do directly with digestion does not appear. It is primarily a blood-forming organ.

Bubonic plague is a disease of the lymphatic system. The great epidemics of fatal type are characterized by the pneumonic and septicemic forms. On the whole, it seems best to place this disease here.

It is evident from the data of Table I that where death ensues from a breakdown of any part of the circulatory or blood systems it is preponderantly the heart itself which is at fault. Diseases of the arteries, which, generally speaking, mean arteriosclerosis, come second in importance. The other causes listed are of relatively minor importance. The relatively enormous rates for diseases of the arteries and for purulent infection and septicemia in São Paulo are noteworthy.

For the present no attempt will be made to discuss the reasons for these differences, since the main object in this

section of the paper is to get the data as a whole before the reader.

The question may fairly be raised as to whether item 22, "Anthrax," should not come in Table I with the blood rather than with the skin in Table VIII. It is a difficult question and one not capable of any absolutely precise solution in the nature of the case. Most fatal cases of anthrax, if not all, are septicemias, or, perhaps better, bacteremias, due finally to failure of the defensive mechanism of the blood. Furthermore, pneumonic and intestinal forms of anthrax occur. On the whole, however, the weight of evidence seems to be that in the majority of cases at least the organism gains its entrance and first victory through the skin, and that the biological strength or weakness of that organ system determines primarily what will subsequently happen. Fortunately, the total rate from anthrax is so small as to be of no significance in any general result.

The causes of death listed in Table II include all of

TABLE II

RESPIRATORY SYSTEM

| No. | "Cause of Death" as per International Classification | Registration Area, U. S. A. | | England and Wales 1914 | Sao Paulo 1917 |
|---|---|---|---|---|---|
| | | 1906–10 | 1901–05 | | |
| 28&29 | Tuberculosis of lungs (including acute miliary tuberculosis) | 146.8 | 170.7 | 104.5 | 119.8 |
| 92 | Pneumonia (lobar and undefined) | 103.0 | 125.5 | 57.5 | 59.9 |
| 91 | Bronchopneumonia | 40.4 | 32.9 | 50.9 | 103.9 |
| 9 | Diphtheria and croup | 22.4 | 29.6 | 16.0 | 9.6 |
| 10 | Influenza | 16.4 | 19.9 | 16.1 | 16.1 |
| 89 | Acute bronchitis | 15.2 | 21.4 | 108.7[8] | 62.1 |
| 8 | Whooping cough | 11.5 | 10.9 | 21.8 | 9.1 |
| 90 | Chronic bronchitis | 11.1 | 15.4 | .... | 3.9 |
| 6 | Measles | 10.8 | 9.0 | 24.7 | 1.5 |
| 94 | Pulmonary congestion and apoplexy | 5.6 | 8.6 | 4.5 | 9.4 |
| 93 | Pleurisy | 4.1 | 4.9 | 4.0 | 7.6 |
| 96 | Asthma | 2.9 | 3.7 | 4.9 | 2.8 |
| 98 | Other respiratory diseases | 2.8 | 4.3 | 1.7 | 5.2 |
| 87 | Diseases of the larynx | 1.7 | 2.3 | 3.2 | 0.9 |
| 97 | Pulmonary emphysema | 0.4 | 0.7 | 1.2 | 2.2 |
| 95 | Gangrene of the lungs | 0.4 | 0.5 | 0.3 | 3.3 |
| 86 | Diseases of the nasal fossae | 0.2 | 0.2 | 0.2 | 0.2 |
| | Totals | 395.7 | 460.5 | 420.2 | 417.5 |

[8] Includes acute and chronic bronchitis.

those under the general heading IV, "Diseases of the respiratory system" of the International Classification, with a single exception, namely No. 88, "Diseases of the thyroid body," which goes elsewhere in the present classification. In addition, there are in Table II four causes of death which are not included with the respiratory system in the International List. These four we may consider in detail.

Item 28, "Tuberculosis of lungs," obviously belongs with the respiratory system, in a strictly organological classification. The breakdown of the lungs as a functioning system is the biological meaning of death from pulmonary tuberculosis. This item is taken from rubric I, "General diseases," of the International Classification. Acute miliary tuberculosis has been included with pulmonary tuberculosis here, rather than as a separate item, for the reason that the English statistics treat these items together. No significant error is introduced by this procedure for two reasons: (*a*) the rate from miliary tuberculosis by itself is very small; and (*b*) probably a majority of cases of acute miliary tuberculosis have the lungs as the chief organ affected.

Item 9, "Diphtheria and croup," is again obviously a respiratory category, on the basis of organs affected. It does not seem to me to be to the point to argue that death in diphtheria is in many cases due to a general toxemia. To do so brings into prominence an aspect of the matter foreign to our present point of view. The infecting agent attacks a part of the respiratory system. If that system were in man as in the insects, lined with chitin in considerable part, presumably death from the clinical entity known as diphtheria would never occur, because the organism would not get the necessary foothold to produce enough toxin to be troublesome. It seems to me further that there is a fundamental biological difference between the cases of scarlet fever and septicemia on the one hand, and diphtheria on the other hand, which leads to the placing of the former with the blood and the latter

with the respiratory system. It is apparent, of course, that the matter of the placing of diphtheria can be argued from both sides, but on the whole I incline to the view that it belongs here with the respiratory organs rather than with the blood.

Item 10, "Influenza," is so obviously respiratory as to require no discussion. The same may be said of item 8, "Whooping cough."

The reason for including item 6, "Measles," here is clearly stated by Spengarn[9] when he says regarding measles: "The mortality of this disease is largely due to the pulmonary complications," and further: "The high mortality among the measles patients in children's hospitals is attributed to bronchopneumonia."

Table II brings out very clearly one important point in favor of the present classification. It is evident from an examination of the four columns of rates that the usages in respect of the diagnostic terminology of respiratory affection, especially the pneumonias and bronchitis, differ greatly in these three countries. Yet the *totals* for all respiratory system deaths are closely similar for all three countries and periods. In other words, the organological totals get rid to a large degree of one of the greatest sources of error in vital statistics, the varying terminology of disease in different regions.

The first and the fourth items in Table III present a new angle of the problem of the classification of the causes of death which needs particular discussion. These items, "Premature birth" and "Injuries at birth" represent a part of the items 151 and 152 of the International Classification. In the International Classification, item 151, which comes under the general heading "XI. Early infancy," has this general title "Congenital debility, icterus and sclerema (total)." This contains two separate subdivisions not numbered, the first being "Premature birth," and the second "Congenital debility,

[9] Spengarn, A., article "Measles" in Ref. Handbook Med. Sci., Vol. VI, p. 283, 1916.

TABLE III

Primary and Secondary Sex Organs

| No. | "Cause of Death" as per International Classification | Registration Area, U. S. A. | | England and Wales 1914 | São Paulo 1917 |
|---|---|---|---|---|---|
| | | 1906–10 | 1901–05 | | |
| 151[11] | Premature birth | 35.7 | 30.8 | 46.9 | 66.8 |
| 42 | Cancer of the female genital organs | 10.8 | 10.0 | 12.9 | 6.5 |
| 137 | Puerperal septicemia | 6.8 | 6.3 | 3.7 | 6.5 |
| 152[11] | Injuries at birth | 6.6 | 5.0 | 2.8 | 2.1 |
| 43 | Cancer of the breast | 6.5 | 5.6 | 10.4 | 1.5 |
| 37 | Syphilis | 5.4 | 4.1 | 5.8 | 15.0[10] |
| 126 | Diseases of the prostate | 3.4 | 2.6 | 4.2 | 0.7 |
| 132 | Salpingitis and other diseases of ♀ genital organs | 2.2 | 2.1 | 0.5 | 0.2 |
| 129 | Uterine tumor (non-cancerous) | 1.8 | 1.8 | 0.8 | 0 |
| 134 | Accidents of pregnancy | 1.7 | 1.7 | 1.1 | 0.2 |
| 130 | Other diseases of the uterus | 1.6 | 1.7 | 0.4 | 0.4 |
| 136 | Other accidents of labor | 1.3 | 0.9 | 1.1 | 0.7 |
| 140 | Following childbirth | 1.1 | 1.5 | 0.1 | — |
| 131 | Cysts and other tumors of ovary | 1.0 | 1.3 | 0.8 | 0.2 |
| 135 | Puerperal hemorrhage | 1.0 | 1.0 | 1.3 | 1.7 |
| 125 | Diseases of the urethra, urinary abscesses, etc. | 0.4 | 0.4 | 1.2 | 0.7 |
| 38 | Gonococcus infection | 0.3 | 0.1 | 0.2 | 0 |
| 128 | Uterine hemorrhage (non-puerperal) | 0.2 | 0.3 | 0 | 0 |
| 127 | Non-venereal diseases of ♂ genital organs | 0.1 | 0.1 | 0.2 | 0 |
| 133 | Non-puerperal diseases of breast (except cancer) | 0.1 | 0.1 | 0.1 | 0 |
| 139 | Puerperal phlegmasia, etc. | 0.1 | — | 0.9 | 0 |
| | Totals | 88.1 | 77.4 | 95.4 | 103.2 |

atrophy, marasmus, etc." Item 152, coming under the same general head of the International Classification has the general title "Other causes peculiar to early infancy (total)." This term contains two unnumbered subdivisions, the first being "Injuries at birth," and the other "Other causes peculiar to early infancy."

The question at once arises, why should these two items "Premature birth" and "Injuries at birth" be included with the primary and secondary sex organs, since it is obvious enough that the infants whose deaths are recorded under these heads in the vast majority of cases, if not all, have nothing whatever the matter with either their primary or secondary sex organs. The answer is,

[10] Including soft chancre (syphilis 1.5, and soft chancre 13.5).

[11] In part. *Cf.* text here.

in general terms, that on any proper biological basis deaths coming under either of these two categories are not properly chargeable organically against the infant at all, but should be charged, on such a basis, against the mother. To go into further detail, it is apparent that when a premature birth occurs it is because the reproductive system of the mother, for some reason or other, did not rise to the demands of the situation of carrying the fetus to term. Premature birth, in short, results from a failure or breakdown in some particular of the maternal reproductive system. This failure may be caused in various ways, which do not here concern us. The essential feature from our present viewpoint is that the reproductive system of the mother does break down, and by so doing causes the death of an infant, and that death is recorded statistically under this title "Premature birth." The death organically is chargeable to the mother.

A considerable number of cases of premature birth are unquestionably due to placental defect and the placenta is a structure of fetal origin, so such deaths could not be properly charged to the mother. On the other hand, however, they would still stay in Table III, because the placenta may fairly be regarded as an organ intimately concerned in reproduction.

The same reasoning which applies to premature births, *mutatis mutandis,* applies to the item "Injuries at birth." An infant death recorded under this head means that some part of the reproductive mechanism of the mother, either structural or functional, failed of normal performance in the time of stress. Usually "injury at birth" means a contracted or malformed pelvis in the mother. But in any case the death is purely external and accidental from the standpoint of the infant. It is organically chargeable to a defect of the sex organs of the mother. The female pelvis, in respect of its conformation, is a secondary sex character.

A practical difficulty arose from the fact that in the São Paulo statistics items 151 and 152 are not subdivided.

In the case of the first of these, item 151, I have ventured to divide the total rate in roughly the same *proportion* between the two subdivisions as exists in the United States and England, namely $\frac{3}{5}$ to premature birth and $\frac{2}{5}$ to congenital debility, etc. While this is admittedly a hazardous proceeding, it seems to me less so than to omit entirely so important a rate, which seems to me the only other practical alternative. In the case of item 152 the total rate is so small (3.3) that no particular difference will be made whatever the basis of distribution used. Consequently, I have again divided it roughly on the basis of the American figures, calling $\frac{5}{8}$ of the total due to injuries at birth.

Table III also includes data which in the International Classification are distributed under three different general heads. First, "General diseases"; second, "Non-venereal diseases of the genito-urinary system and annexa"; and third, "Puerperal state." In the International List all cancers are included under "General diseases." We have taken out for inclusion here the several cancers of the primary and secondary sex organs, including item 42, "Cancer of the female genital organs," and item 43, "Cancer of the breast." Items 37 and 38, "Syphilis" and "Gonococcus infection," are also taken out of the class of "General diseases" of the International List. The immediate reason for including these diseases here is obvious, but particularly in relation to syphilis the point at once needs further discussion. As a cause of actual death, syphilis frequently acts through the central nervous system, and the question may fairly be raised why, in view of this fact, syphilis is not there included. The point well illustrates one of the fundamental difficulties in any organological classification of disease. In the case of syphilis, however, the difficulty in practise is not nearly so great as it is in theory. As a matter of fact, most of the deaths from the effect of syphilitic infection on the nervous system are recorded in vital statistics by reporting physicians and vital statis-

ticians as diseases of the nervous system. For example, it is perfectly certain that most of the deaths recorded as due to "locomotor ataxia" and "softening of the brain" are fundamentally syphilitic in origin. The rate included in Table III of 5.4 for the Registration Area of the United States in 1906–10 for deaths due to syphilis is far lower, as any clinician knows, than the number of deaths really attributable to syphilitic infection. These other deaths, due to syphilis, and not reported under that title, are reported under the organ which primarily breaks down and causes death, as, for example, the brain, and will in the present system of classification be included under the nervous system. After careful consideration it has seemed as fair and just as anything which could be done to put the residue of deaths specifically reported as due to syphilis under Table III, Primary and Secondary Sex Organs. The rate in any event is so small that whatever shift was made could not sensibly affect the general results to which we shall presently come.

The question may be asked as to why puerperal septicemia (item No. 137) is included here and not with the diseases of the circulatory system and blood on the same reasoning that general septicemia was put there. The cases seem to be essentially different. Puerperal septicemia arises fundamentally because of a failure of the reproductive system of the female to meet in a normal way the demands made upon it by the process of reproduction itself. In line with the general reasoning on which we are working in this classification, it would therefore seem that this cause of death belongs where it has been put here, with the primary and secondary sex organs. The same sort of reasoning applies to the other puerperal causes of death here included.

Item 125, "Diseases of the urethra, urinary abscesses, etc." is placed with the sex organs rather than with the excretory organs in Table IV, because, with very few exceptions, the deaths in this item are sequelæ of gonorrhea. Urinary abscesses are secondary usually to urethral

stricture, which in turn, except for an insignificant number of traumatic cases, is gonorrheal in origin.

Regarding the wisdom of bringing together under one rubric the causes of death listed in Table IV on a biological basis, there would seem to be little doubt with a single exception. This does present a very difficult problem. Item 138, Puerperal albuminuria, is included here rather than with other puerperal diseases under the sex organs, or elsewhere, on the reasoning that the cause of death is finally the organic breakdown of the kidneys and not of the reproductive system, and bespeaks a fundamental organic weakness of the excretory system, which weakness is made to flare up into clinical nephritic trouble under the strain of pregnancy. Basically these toxemias are due to faulty maternal metabolism, of unknown origin, which can not in the present state of ignorance be properly charged against any particular organ or organ system. It, however, remains a fact that many women having organically sound excretory organs are able to weather even very severe metabolic storms of this sort near the end of pregnancy and survive. Others with organically weak excretory systems go down. In view of these facts it seems on the whole fairer to put these deaths here than against any other organ system.

TABLE IV

KIDNEYS AND RELATED EXCRETORY ORGANS

| No. | "Cause of Death" as per International Classification | Registration Area, U.S.A. | | England and Wales | Sao Paulo |
|---|---|---|---|---|---|
| | | 1906–10 | 1901–05 | 1914 | 1917 |
| 120 | Bright's disease | 87.4 | 87.4 | 37.0 | 41.2 |
| 119 | Acute nephritis | 10.1 | 9.6 | 5.4 | 29.4 |
| 138 | Puerperal albuminuria and convulsions | 3.4 | 2.8 | 1.7 | 1.7 |
| 124 | Diseases of the bladder | 3.1 | 4.3 | 3.3 | 1.3 |
| 121 & 122 | Chyluria and other diseases of the kidneys | 2.6 | 2.8 | 1.3 | 9.4 |
| 123 | Calculi of the urinary passage | 0.6 | 0.5 | 0.7 | 0.4 |
| | Totals | 107.2 | 107.4 | 49.4 | 83.4 |

The "rheumatisms" present another difficult ques-

tion. A precise and critical decision on the point of where these diseases belong in this present scheme of classification is impossible of attainment. Weighing all the evidence carefully, it seemed best to put chronic rheumatism and gout and acute articular rheumatism in Table V, under "Skeletal and muscular system," rather than here with the kidneys. Much at least of the fatal chronic rheumatism is really a chronic infective arthritis. Gout is a disease due to fundamental disturbances of general metabolism, but the statistical returns lump deaths from this cause with chronic rheumatism. The death rates from all of these diseases are, fortunately, so small that it makes no essential difference to the final synthetic result towards which we are working where they are placed.

TABLE V

SKELETAL AND MUSCULAR SYSTEM

| No. | "Cause of Death" as per International Classification | Registration Area, U. S. A. | | England and Wales | São Paulo |
|---|---|---|---|---|---|
| | | 1906–10 | 1901–05 | 1914 | 1917 |
| 47 | Acute articular rheumatism | 5.4 | 5.2 | 5.6 | 2.6 |
| 146 | Diseases of the bones | 2.5 | 2.4 | 1.5 | 0.7 |
| 48 | Chronic rheumatism and gout | 2.2 | 3.6 | 5.4 | 0 |
| 32 | Pott's disease | 1.5 | 1.5 | 1.6 | 2.6 |
| 33 | White swellings | 0.7 | 0.7 | 0.9 | 0 |
| 147 | Diseases of the joints | 0.2 | 0.2 | 0.4 | 0 |
| 149 | Other diseases of the organs of locomotion | 0.1 | 0.1 | 0.1 | 0 |
| 36 | Rickets | [12] | [12] | 2.7 | 0.9 |
| | Totals | 12.6 | 13.7 | 18.2 | 6.8 |

Item 47, "Acute articular rheumatism," and the two tubercular affections, items 32 and 33 (Pott's disease and white swellings) are placed here because the essential lesion produced by the causative agents is in either the bones or the joints.

All of the rates in Table V are small, and any of the causes of death listed therein could be shifted to other rubrics without sensibly affecting any general result.

[12] Not separately tabulated.

In Table VI are included a number of causes of death beyond those which are included in general heading "V. Diseases of the digestive system" in the International

TABLE VI

Alimentary Tract and Associated Organs Concerned in Metabolism

| No. | "Cause of Death" as per International Classification | Registration Area, U. S. A. 1906–10 | Registration Area, U. S. A. 1901–05 | England and Wales 1914 | São Paulo 1917 |
|---|---|---|---|---|---|
| 104 | Diarrhea and enteritis (under 2) | 96.2 | 89.0 | 63.6[14] | 383.6 |
| 151[13] | Congenital debility, atrophy, marasmus | 28.8 | 23.2 | 27.1 | 44.3 |
| 40 | Cancer of the stomach and liver | 28.3 | 24.7 | 36.5 | 28.1 |
| 1 | Typhoid fever | 25.6 | 32.0 | 4.6 | 14.4 |
| 103 | Other diseases of stomach | 16.8 | 17.7 | 10.9 | 1.3 |
| 105 | Diarrhea and enteritis (2 and over) | 16.7 | 20.2 | — | 49.9 |
| 113 | Cirrhosis of the liver | 14.3 | 14.4 | 11.2 | 12.2 |
| 50 | Diabetes | 13.7 | 11.5 | 12.2 | 5.4 |
| 109 | Hernia and intestinal obstruction | 12.9 | 13.0 | 10.9 | 3.0 |
| 71 | Convulsions of infants | 12.5 | 21.4 | 22.7 | 10.0 |
| 108 | Appendicitis and typhlitis | 11.2 | 11.0 | 7.1 | 3.5 |
| 41 | Cancer of the peritoneum, intestines, rectum | 8.8 | 7.1 | 21.3 | 4.1 |
| 14 | Dysentery | 6.5 | 8.6 | 0.7 | 9.6 |
| 117 | Simple peritonitis | 6.1 | 10.8 | 1.4 | 13.3 |
| 115 | Other diseases of the liver | 6.1 | 7.5 | 2.6 | 5.7 |
| 31 | Abdominal tuberculosis | 6.0 | 6.0 | 9.4 | 1.7 |
| 150[13] | Other congenital malformations | 4.5 | 3.9 | 5.4 | [15] |
| 102 | Ulcer of the stomach | 3.6 | 2.9 | 5.5 | 3.5 |
| 110 | Other diseases of intestines | 2.8 | 2.9 | 1.4 | 5.4 |
| 114 | Biliary calculi | 2.8 | 2.2 | 2.3 | 0.4 |
| 39 | Cancer of the buccal cavity | 2.6 | 2.1 | 6.6 | 1.3 |
| 35 | Disseminated tuberculosis | 2.5 | 2.8 | 5.5 | 2.2 |
| 100 | Diseases of the pharynx | 1.6 | 1.4 | 2.1 | 0.4 |
| 13 | Cholera nostras | 1.0 | 1.4 | 0.1 | 0.2 |
| 99 | Diseases of the mouth | 0.7 | 0.7 | 1.5 | 0.4 |
| 59 | Other chronic poisonings | 0.5 | 0.5 | 0 | 0.2 |
| 118 | Other diseases of the digestive system | 0.5 | 0.3 | 0.6 | 1.1 |
| 111 | Acute yellow atrophy of liver | 0.4 | 0.4 | 0.2 | 0.4 |
| 101 | Diseases of the esophagus | 0.3 | 0.3 | 0.2 | 1.3 |
| 57 | Chronic lead poisoning | 0.2 | 0.3 | 0.2 | 0 |
| 26 | Pellagra | 0.2 | 0 | 0 | 0 |
| 49 | Scurvy | 0.1 | 0.1 | 0.1 | 0.2 |
| 106 & 107 | Parasites | 0.1 | 0.1 | 0.1 | 6.3 |
| 112 | Hydatid tumor of liver | [16] | [16] | 0.1 | 0.2 |
| 27 | Beriberi | [17] | [17] | 0 | 0.2 |
| 12 | Asiatic cholera | 0 | 0 | 0 | 0 |
| | Totals | 334.9 | 340.4 | 274.1 | 613.8 |

[13] In part.

[14] Diarrhea and enteritis, all ages.

[15] See footnote to Table I.

[16] Death rate less than 0.1 per 100,000.

[17] Not separately tabulated for period named.

Classification. Of these causes which have been brought in from other parts of the International Classification the first which demands attention is the second on the list "Congenital debility, atrophy, marasmus." This is a part of item No. 151 of the International Classification. As already pointed out, that item includes "Premature birth," which has in the present classification been placed under "Primary and secondary sex organs" for reasons already stated, and "Congenital debility, atrophy, marasmus, etc.," which is the part included here. The reason for putting this portion of item 151 under the present heading is the practical one that clinical experience shows that the vast majority of the deaths of infants which are statistically recorded under this heading "Congenital debility, atrophy, and marasmus" are actually due to deficiencies, functional, structural, or both, in the alimentary tract. In probably more than 95 per cent. of all cases "Congenital debility" of an infant means that something is wrong with the alimentary tract in its immediate metabolic functions.

Item 50, "Diabetes," includes deaths from a disease which, while diagnosed from a disarrangement of the excretory function, is primarily an affection of the organs which have to do with the initial or early stages of metabolism (the liver, the pancreas, etc.). It therefore seems to belong properly in the classification where it is now placed rather than with the kidneys. In the International Classification it is included with "General diseases."

Item 71, "Convulsions of infants," is in the International Classification placed with "Diseases of the nervous system." It is transferred from that location to the present one in this classification because of the well-known clinical fact that the vast majority of deaths of infants recorded as due to convulsions are really due to profound disarrangements of the alimentary tract, which eventually lead to convulsions. Biologically, the fundamental breakdown in such cases is of the alimen-

tary tract and associated organs, and not of the brain or central nervous system.

The part of item 150 of the International Classification bearing the title "Other congenital malformations" needs some discussion in regard to its inclusion here. In other rubrics of the present classification we have taken account of hydrocephalus and congenital malformation of the heart, both of which come under the general heading "X. Malformations" of the International Classification. The only other rubric under that heading in the International Classification is the one here under discussion "Other congenital malformations." It is, of course, impossible to say in detail what these other congenital malformations are. It seems fair, however, to assume from general knowledge that after hydrocephalus and congenital malformations of the heart are deleted, the great majority of the remaining congenital malformations will relate directly to the alimentary tract or some of its associated organs. Quantitative proof that this is the case is not forthcoming for obvious reasons. The placing of this item here is simply on the basis of the best information it is possible to get from those most familiar with congenital malformations in infants. There is undoubtedly some error inherent in placing this title here, but the net effect of such error must be insignificant for the reason that the death rate under this rubric is very small in total, as will be seen from the table, and furthermore, as has already been stated, it is certain on general grounds that the vast bulk of deaths included here must be due to malformations of the alimentary tract or its associated organs.

Items No. 31 and 35 (Abdominal tuberculosis, and disseminated tuberculosis) are placed here, because, while these titles are somewhat indefinite, it is quite certain that the major portion of the deaths recorded by health officers under these terms are due to tubercular affections of the alimentary tract.

Items 57, 59, 26, 27, and 49 (chronic lead poisoning,

other chronic poisonings, pellagra, beriberi, and scurvy) present an interesting problem. The question is whether they should go here or with external causes in Table X. It can be argued that on the one hand, the poisonings are due simply to the ingestion of a deleterious agent and death has no biological basis any more than if a person is struck by an automobile, and, on the other hand, that deaths from the diseases like pellagra and beriberi again simply arise from the fact that the victim lacked a proper diet. But the case is not so simple as this argument would imply. Not all workers in paint factories, nor all inmates of insane asylums or prisons die from these causes. Some survive. And it is reasonable, it seems to me, to suppose that in many cases at least the determining factor in the survival is the relative organic soundness or "strength" of the organs primarily concerned in metabolism. On this basis, this group of causes of death is included in Table VI. Fortunately, they are all insignificant contributions to the total death rate.

Regarding the other items in Table VI, taken from the "General disease" class of the International Classification, there is no need for discussion because it is sufficiently evident that on a biological classification they belong here rather than with any other organ group.

The enormous excess of the Sao Paulo death rate for the total of the items in Table VI as compared with the Registration Area of the United States and England and Wales is noteworthy. Examination of the data will show that it arises almost entirely from the excessive death rate in Sao Paulo from diarrhea and enteritis (under 2).

In the main the causes of death included in Table VII in addition to those which appear in class II, "Diseases of the nervous system and of the organs of special sense" of the International Classification, so obviously belong here as to require no special discussion. Two, however, call for comment. Of these the most important is suicide. In the International Classification suicides are placed under "XIII. External causes," a singularly inept loca-

TABLE VII

NERVOUS SYSTEM AND SENSE ORGANS

| No. | "Cause of Death" as per International Classification | Registration Area, U.S.A. 1906–10 | Registration Area, U.S.A. 1901–05 | England and Wales 1914 | São Paulo 1917 |
|---|---|---|---|---|---|
| 64 | Cerebral hemorrhage and apoplexy | 71.7 | 69.6 | 65.3 | 32.9 |
| 61 | Meningitis (total) | 19.4 | 31.7 | 11.5 | 43.1 |
| 66 | Paralysis without specified cause | 16.1 | 20.1 | 7.3 | 2.6 |
| | Suicide (total) | 16.0 | 13.9 | 10.0 | 12.9 |
| 30 | Tuberculous meningitis | 9.1 | 8.9 | 12.6 | 0 |
| 56 | Alcoholism | 5.8 | 6.1 | 1.8 | 2.8 |
| 63 | Other diseases of the spinal cord | 5.8 | 4.9 | 7.5 | 4.4 |
| 73 & 74 | Neuralgia, neuritis and other diseases of the nervous system | 5.5 | 6.9 | 7.0 | 2.0 |
| 67 | General paralysis of the insane | 5.5 | 6.8 | 6.1 | 3.0 |
| 69 | Epilepsy | 4.2 | 4.4 | 7.6 | 4.8 |
| 68 | Other forms of mental alienation | 3.6 | 3.6 | 2.7 | 0.7 |
| 24 | Tetanus | 2.7 | 3.5 | 0.5 | 4.6 |
| 62 | Locomotor ataxia | 2.6 | 2.4 | 1.9 | 0.2 |
| 65 | Softening of the brain | 2.5 | 3.7 | 3.9 | 0.7 |
| 76 | Diseases of the ears | 1.6 | 1.3 | 3.3 | 0 |
| 150[18] | Hydrocephalus | 1.4 | 1.6 | 1.0 | [19] |
| 60 | Encephalitis | 1.1 | 1.9 | 0.9 | 1.3 |
| 70 | Convulsions (non-puerperal) | 0.5 | 1.1 | 0.3 | 1.7 |
| 72 | Chorea | 0.2 | 0.3 | 0.5 | 0 |
| 23 | Rabies | 0.2 | 0.1 | 0 | 0.7 |
| 75 | Diseases of the eye and annexa | 0.1 | 0.1 | 0.2 | 0 |
| 17 | Leprosy | [20] | [20] | 0 | 5.9 |
| | Totals | 175.6 | 192.9 | 151.9 | 124.3 |

tion biologically. The immediate motivation of a suicidal death is surely internal. A searching biological analysis of the phenomenon of suicide has yet to be made, but certain of its biological relations are clear enough. In the broadest terms people commit suicide because their higher cerebral mechanism breaks down under the stresses of the world in which they live, and fails to continue its normal functioning. One of the deepest rooted instincts of the individual among all living things, from lowest to highest, is the instinct for the preservation of the individual life. The only instinct which transcends it, and that only in comparatively few cases in lower animals, is the instinct of reproduction. But the phenom-

[18] In part.

[19] See footnote Table I.

[20] Less than 0.1 per 100,000.

enon of suicide in man marks the complete and total inhibition of this instinct of self-preservation. Suicide is always an act in some degree mentally deliberated before its performance. A constitutionally and hygienically sound mentality weathers the environmental storm which suggests suicide. On the basis of this reasoning suicide death rate is put in Table VII.

Item 56, "Alcoholism," is included here because fundamentally deaths so returned would seem to be more truly chargeable against the central nervous system than to any other organ system. This opinion is founded on such results as those of Barrington and Pearson,[21] who conclude, after a careful analysis of data regarding extreme and chronic inebriates, that "there appears for constant age little relation between alcoholism and physical fitness," while between mental defect (and poor education) and alcoholism there is a sensible relation. "We consider it probable . . . that the alcoholism is not due to the poor education, nor is it to any marked extent productive of the mental defect, but the want of will-power and self-control associated with the mental defectiveness is itself the antecedent of the poor education and of the alcoholism."

The other cause of death needing special comment here is leprosy. I am informed by my friend, Dr. G. H. de Paula Souza, who has had unusual opportunities to know leprosy in all its clinical manifestations, that when this disease becomes fatal it is the nervous system which disintegrates and leads to death.

The first five items in Table 8 are affections of the skin about which there can be no doubt respecting the correctness of their inclusion here. The last four items, smallpox, anthrax, mycoses and glanders are all diseases with very low death rates at the present time. Biologically, they represent diseases which either gain entrance through the skin, or in which the principal lesions are of

[21] Barrington, A., and Pearson, K., "A Preliminary Study of Extreme Alcoholism in Adults," *Eugenics Lab. Mem.*, XIV, 1910.

TABLE VIII

The Skin

| No. | "Cause of Death" as per International Classification | Registration Area, U. S. A. 1906–10 | Registration Area, U. S. A. 1901–05 | England and Wales 1914 | São Paulo 1917 |
|---|---|---|---|---|---|
| 18 | Erysipelas | 4.2 | 4.5 | 3.1 | 1.3 |
| 44 | Cancer of the skin | 2.7 | 2.3 | 2.6 | 1.5 |
| 144 | Acute abscess | 1.1 | 1.4 | 2.1 | 0.7 |
| 145 | Other diseases of the skin | 1.0 | 1.0 | 3.4 | 2.4 |
| 143 | Furuncle | 0.5 | 0.5 | 0.7 | 0.2 |
| 5 | Smallpox | 0.2 | 3.4 | 0 | 0.7 |
| 22 | Anthrax | 0.2 | 0.1 | 0 | 0 |
| 25 | Mycoses | 0.2 | — | 0.1 | 1.1 |
| 21 | Glanders | — | 0.1 | 0 | 0 |
| | Totals | 10.1 | 13.3 | 12.0 | 7.9 |

the skin. It therefore appears that on the present scheme of classification they may best be put here.

There is no doubt whatever that the three diseases of Table IX belong biologically with the endocrinal system.

In the foregoing tables have been included all statistically recognized causes of death which it is now possible to classify on an organological basis, and which have a

TABLE IX

Endocrinal System

| No. | "Cause of Death" as per International Classification | Registration Area, U.S.A. 1906–10 | Registration Area, U.S.A. 1901–05 | England and Wales 1914 | São Paulo 1917 |
|---|---|---|---|---|---|
| 51 | Exophthalmic goiter | 1.1 | 0.7 | 1.3 | 0.4 |
| 52 | Addison's disease | 0.4 | 0.5 | 0.6 | 0.7 |
| 88 | Diseases of the thyroid body | 0.4 | 0.3 | 0.8 | 0 |
| | Totals | 1.9 | 1.5 | 2.7 | 1.1 |

significant death rate. The residue comprises in general three categories (*a*) accidental and homicidal deaths; (*b*) senility; and (*c*) deaths from a variety of causes which are statistically lumped together and can not be disentangled. Accidental and homicidal deaths find no place in a biological classification of mortality. A man organically sound in every respect may be instantly killed by being struck by a railroad train or an automo-

bile. The best possible case that could be made out for a biological factor in such deaths would be that contributory carelessness or negligence, which is a factor in some portion of accidental deaths, bespeaks a small but definite organic mental inferiority or weakness, and that, therefore, accidental deaths should be charged against the nervous system. This, however, is obviously not sound. For in the first place in many accidents there is no factor of contributory negligence in fact, and in the second place in those cases where such negligence can fairly be alleged its degree or significance is undeterminable and in many cases surely slight.

Senility as a cause of death is not further classifiable on an organological basis. A death really due to old age, in the sense of Metchnikoff, represents, from the point of view of the present discussion, a breaking down or wearing out of all the organ systems of the body contemporaneously. In a strict sense this probably never, or at best extremely rarely, happens. But physicians and registrars of mortality still return a certain number of deaths as due to "senility." Under the circumstances it is not possible to go behind such returns biologically.

TABLE X

ALL OTHER CAUSES

| No. | "Cause of Death" as per International Classification | Registration Area, U. S. A. | | England and Wales 1914 | São Paulo 1917 |
|---|---|---|---|---|---|
| | | 1906–10 | 1901–05 | | |
| | All external causes (except suicide) | 91.9 | 87.8 | 26.1 | 36.4 |
| 187, 188 & 189 | Ill-defined diseases | 29.4 | 47.8 | 7.3 | 36.3 |
| 154 | Senility | 29.0 | 41.0 | 81.5 | 11.1 |
| 45 | Cancer of other organs or of organs not specified | 12.9 | 16.1 | 16.6 | 17.9 |
| 152[22] | Other causes peculiar to early infancy | 3.4 | 2.6 | 5.1 | 3.3 |
| 34 | Tuberculosis of other organs | 2.1 | 2.0 | 1.6 | 0.2 |
| 46 | Other tumors (female genital organs excepted) | 1.0 | 1.5 | 0.5 | 0.9 |
| 55 | Other general diseases | 1.0 | 0.5 | 1.5 | 3.5 |
| 153 | Lack of care | 0.3 | 12.3 | 0.6 | 0 |
| 19 | Other epidemic diseases | 0.3 | 0.2 | 0.6 | 0.2 |
| | Totals | 171.3 | 211.9 | 141.4 | 109.8 |

[22] Less than 0.1 per 100,000.

The second line of Table X, "Ill-defined diseases," furnishes a striking commentary on the relative efficiency of the medical profession in the United States and England in respect of the reporting of the causes of death. Only about one fourth as many deaths appear in the English vital statistics as due to ill-defined and unknown causes as in the United States figures. Happily, the conditions in this regard are constantly improving in the Registration Area of the United States, due to the well-conceived and untiring efforts of the officials in charge of vital statistics in the Bureau of the Census. They deserve the warmest gratitude of every American vital statistician for the improvements in registration they have brought about.

## IV

Having now arranged, so far as possible, all statistically recognized causes of death in a biological classification, we may turn to an examination of the results which such an arrangement shows. In Table XI the totals of Tables I to IX, inclusive, are arranged in descending order of magnitude. The results are shown graphically in Fig. 1.

TABLE XI

SHOWING THE RELATIVE IMPORTANCE OF DIFFERENT ORGAN SYSTEMS IN HUMAN MORTALITY

| Group No. | Organ System | Death Rates per 100,000 | | | |
|---|---|---|---|---|---|
| | | Registration Area, U. S. A. | | England and Wales | São Paulo 1917 |
| | | 1906-10 | 1901–05 | 1914 | |
| II | Respiratory system | 395.7 | 460.5 | 420.2 | 417.5 |
| VI | Alimentary tract and associated organs | 334.9 | 340.4 | 274.1 | 613.8 |
| I | Circulatory system, blood | 209.8 | 196.8 | 208.6 | 254.8 |
| VII | Nervous system and sense organs | 175.6 | 192.9 | 151.9 | 124.3 |
| IV | Kidneys and related excretory organs | 107.2 | 107.4 | 19.4 | 83.4 |
| III | Primary and secondary sex organs | 88.1 | 77.4 | 95.4 | 103.2 |
| V | Skeletal and muscular system | 12.6 | 13.7 | 18.2 | 6.8 |
| VIII | Skin | 10.1 | 13.3 | 12.0 | 7.9 |
| IX | Endocrinal system | 1.5 | 1.2 | 1.9 | 1.1 |
| | Total death rate classifiable on a biological basis | 1,335.5 | 1,403.6 | 1,201.7 | 1,612.8 |
| X | All other causes of death | 171.3 | 211.9 | 141.4 | 109.8 |

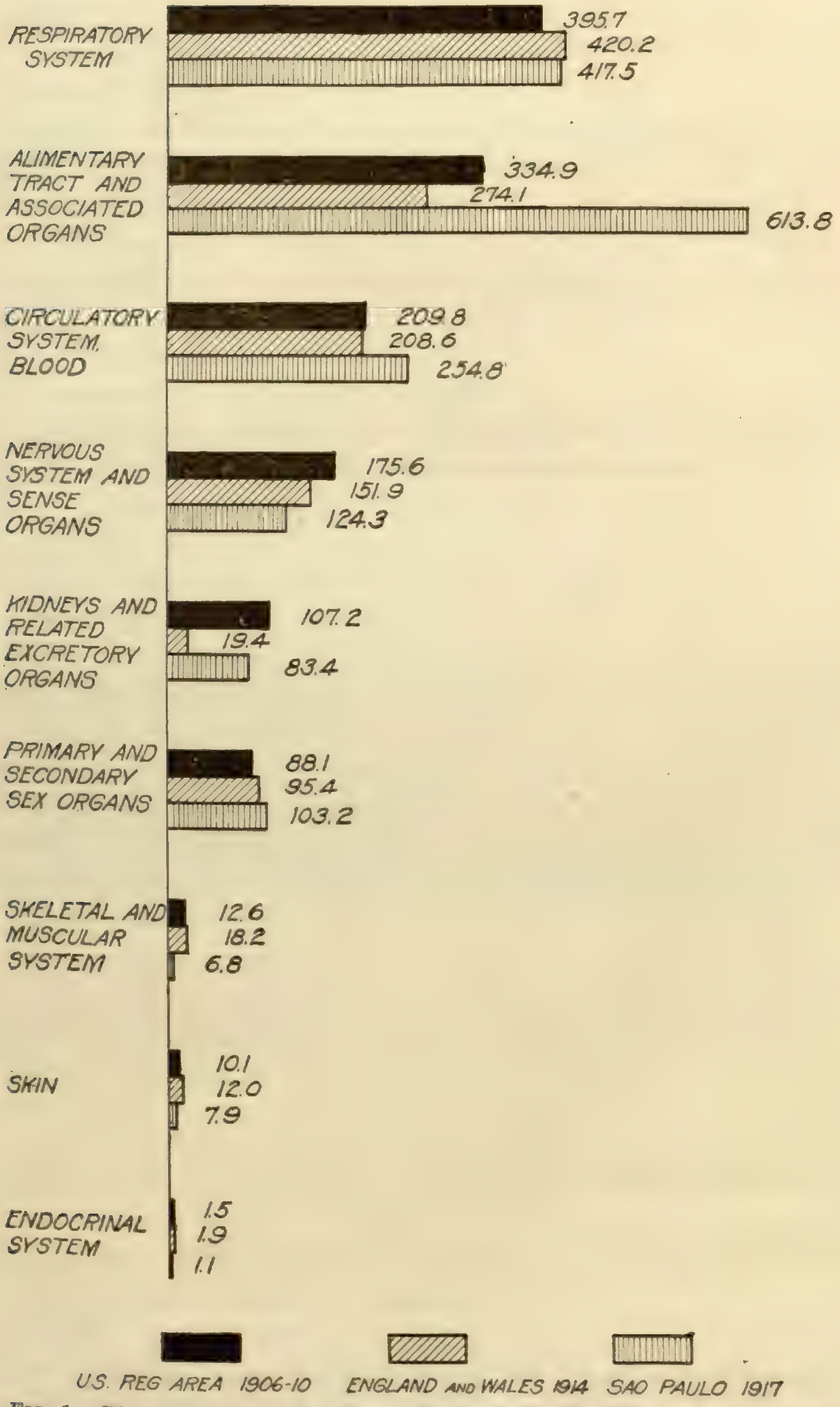


FIG. 1. Diagram showing the relative importance of the different organ systems of the body in human mortality.

From Table XI and the diagram a number of noteworthy points can be made out.

1. In the United States, during the decade covered, more deaths resulted from the breakdown of the respiratory system than from the failure of any other organ system of the body. The same thing is true of England and Wales. In São Paulo the alimentary tract takes first position, with the respiratory system a rather close second. The tremendous death rate in Sao Paulo chargeable to the alimentary tract is chiefly due, as Table VI clearly shows, to the relatively enormous number of deaths of infants under two from diarrhea and enteritis. Nothing approaching such a rate for this category as Sao Paulo shows is known in this country or England.

2. In all three localities studied the respiratory system and the alimentary tract together account for rather more than half of all the deaths biologically classifiable. These are the two organ systems which, while physically internal, come in contact directly at their surfaces with environmental entities (water, food, and air) with all their bacterial contamination. The only other organ system directly exposed to the environment is the skin. The alimentary canal and the lungs are, of course, in effect invaginated *surfaces* of the body. The mucous membranes which line them are far less resistant to environmental stresses, both physical and chemical, than is the skin with its protecting layers of stratified epithelium.

3. The organs concerned with the blood and its circulation stand third in importance in the mortality list. Biologically the blood, through its immunological mechanism, constitutes the second line of defense which the body has against noxious invaders. The first line is the resistance of the outer cells of the skin and the lining epithelium of alimentary tract, lungs, and sexual and excretory organs. When invading organisms pass or break down these first two lines of defense the battle is then with the home guard, the cells of the organ system which, like the industrial workers of a commonwealth, keep the body

going as a whole functioning mechanism. Naturally it would be expected that the casualties would be far heavier in the first two defense lines (respiratory and alimentary systems, and blood and circulation) than in the home guard. Death rates when biologically classified bear out this expectation.

4. It is at first thought somewhat surprising that the breakdown of the nervous system is responsible for more deaths than that of the excretory system. When one bears in mind, however, the relative complexity of the two pieces of machinery, it is perceived that the relative position of the two in responsibility for mortality is what might reasonably be expected.

5. In the United States the kidneys and related excretory organs are responsible for more deaths than the sex organs. This relation is reversed in England and Wales and in Sao Paulo. A return to Table III shows that this difference is mainly due, in the case of England, to two factors, premature birth and cancer. In Sao Paulo it is due to premature birth and syphilis. The higher premature birth rate for these two localities as compared with the United States might conceivably be explained in either of two ways. It might mean better obstetrics here than in the other localities, or it might mean that the women of this country as a class are somewhat superior physiologically in the matter of reproduction, when they do reproduce. The first suggestion seems definitely more improbable than the second. The higher apparent syphilis rate of Sao Paulo probably means nothing more than better reporting, a less prudish disinclination to report syphilis as a cause of death in Sao Paulo than in the other two countries. It is by no means beyond the bounds of possibility that if all deaths really due to syphilis and gonorrhea were actually reported as such, the rate for the sex organs would be decidedly higher than for the excretory organs in all countries.

6. The last three organ systems in the table, skeletal and muscular system, skin and endocrinal organs, are

responsible for so few deaths relatively as not to be of serious moment.

7. In a broad sense the efforts of public health and hygiene have been directed against the affections comprised in the first two items in the table, respiratory system and alimentary tract. The figures in the first two columns for the two five-year periods in the United States indicate roughly the rate of progress such measures are making, looking at the matter from a broad biological standpoint. In reference to the respiratory system there was a decline of 14 per cent. in the death rate between the two periods. This is substantial. It is practically all accounted for in phthisis, lobar pneumonia and bronchitis. For the alimentary tract the case is not so good —indeed, far worse. Between the two periods the death rate from this cause group fell only 1.8 per cent. Reference to Table VI shows how all the gain made in typhoid fever was a great deal more than offset by diarrhea and enteritis (under two), congenital debility and cancer. Child welfare, both prenatal and postnatal, seems by long odds the most hopeful direction in which public health activities can expect at the present time substantially to reduce the general death rate. This is a matter fundamentally of education. Ignorant and stupid people must be taught, gently if possible, forcibly if necessary, how to take care of a baby both before and after it is born. It seems at present unlikely that mundane law will regard feeding a two months old baby cucumber, or dispensing milk reeking with deadly poison makers, as activities accessory to first-degree murder. But we are moving in that direction under the enthusiastic and capable leadership of the Federal Children's Bureau. And there is this further comfort, that if that final Judgment Seat, before which so many believe we must all eventually appear, dispenses that even-handed justice which in decency it must, many of our most prominent citizens who in the financial interests of themselves or their class block every move towards better sewage disposal, water and milk

supply and the like, or force pregnant women to slave over washtub or sewing bench that they may live, will find themselves irrevocably indicted for the wanton and wilful slaughter of innocent babies.

## V

We come now to the final stage in this study. Having arranged so far as possible all causes of death on an organological base, it occurred to me to go one step further back and combine them under the headings of the primary germ layers from which the several organs developed embryologically. To do this is a task of considerable difficulty. It raises intricate, and in some cases still unsettled, questions of embryology. Furthermore, the original statistical rubrics under which the data are compiled by registrars of vital statistics were never planned with such an object as this in mind. Still the thing seemed worth trying because of the evolutionary interest which would attach to the result, even though it were somewhat crude and in respect of minor and insignificant details open to captious criticism.

In Tables XII and XIII the death rates of Tables I to IX are subsumed under the three captions, ectoderm, mesoderm and endoderm, according as the organ concerned developed from one or the other of these germ layers. It will be necessary, however, before presenting the tables, to set forth in detail how the figures they contain were made up.

*A. Ectoderm.*—Under this head were placed first, in making up Table XII, the totals of Table VII (the nervous system and sense organs), and Table VIII (the skin). To the sum obtained by adding these totals together was added (*a*) item 39 (cancer of the buccal cavity) from Table VI, on the ground that the lining of the buccal cavity is ectodermal in origin; (*b*) 0.30 of the rates under item 41 (cancer of the peritoneum, intestines and rectum). The point here was that the lining epithelium of the rectum is derived from ectoderm. The cancer rates for

these three embryologically different organs, rectum, intestines and peritoneum are arbitrarily lumped together by the registrars of vital statistics. It is necessary for present purposes to unscramble the figures with as little arbitrariness as possible. Data (admittedly rather meager) given by Hoffman[23] (pp. 116–121) from the New York State investigation indicate that in a lumped total of cancer of the peritoneum, intestines and rectum, the fractions incident upon each of the organs are about 0.04 for peritoneum, 0.30 for rectum, and 0.66 for intestines. As these figures are much less arbitrary than a mere guess, I have adopted them. It should be remembered that in the final result it makes little difference what fraction is adopted, because the total rate under item 41 is so small. A remarkable thing which comes out when the lumped figures for cancer of peritoneum, intestines and rectum are subdivided in the above-named proportions, is the similarity, amounting practically to identity, in the death rates in all four times and places studied, from cancer of the buccal cavity and cancer of the rectum. The figures are as follows:

| | U. S. A. | | England and Wales | São Paulo |
|---|---|---|---|---|
| | 1906–10 | 1901–05 | | |
| Cancer of buccal cavity | 2.6 | 2.1 | 6.6 | 1.3 |
| Cancer of rectum (calculated) | 2.6 | 2.1 | 6.4 | 1.2 |

This identity can hardly be accidental, since it occurs in all three different localities with quite different cancer rates in each. It indicates that the fundamental embryological likeness between buccal cavity and rectum is accurately reflected in their neoplastic pathology, provided it can be safely assumed the portion of the rectum in which cancers preponderantly occur is ectodermic. This appears to be the fact. (*c*) Item 86 (diseases of the nasal fossæ) is added, because the lining membrane of the nose is ectodermal in origin.

[23] Hoffman, F. L., "The Mortality from Cancer throughout the World," Newark, 1915.

*B. Mesoderm.*—Here the figures of Table XII were reached by the following process. First, the totals of Table I (circulatory system), Table III (sex organs), and Table IV (kidneys), were added together, these being obviously in general mesodermic. From the total so obtained was *subtracted* item 124 of Table IV (diseases of the bladder) since the lining epithelium, the most vulnerable part pathologically, is endodermic in origin. For the same reason item 125 of Table IV (diseases of the urethra) was next subtracted. To the result so obtained was *added* (*a*) the total of Table V (skeletal system) and item 52 (Addison's disease) from Table IX, these representing organs mesodermic in origin; (*b*) 0.04 of the rate under item 41 of Table VI (cancer of peritoneum); (*c*) item 117 (simple peritonitis); (*d*) item 93 (pleurisy). The pleura and peritoneum are mesodermic structures and therefore clearly belong here. The final totals reached after the above described process are those which appear under "Mesoderm" in Table XII.

Up to this point in the argument it has been assumed, without discussion, that all the items in Table VII (the nervous system and sense organs) go with the ectoderm. There is, however, another point of view possible here, which may be stated in the following way. Cerebral hemorrhage and apoplexy (item 64) and softening of the brain (item 65) are brain conditions brought about by a precedent functional breakdown of a part of the vascular system, namely the terminal arteries of the brain. Cerebral hemorrhage is due to the rupture of an artery or arteries in the brain, and may in and of itself be a sufficient cause of death, just as would be a hemorrhage due to rupture of an artery in any other part of the body. So far as anything now known can tell us, this fatal accident is as likely as not to occur in a brain of which the nerve cells (of ectodermic origin) are perfectly sound organically. Should such a death be charged against the ectoderm? The case is at least open to question.

It might at first be supposed that the same argument would justify the placing of cerebral hemorrhage with the circulatory system in the primary organological classification, but this does not seem to be warranted. From an organological point of view the brain must be considered as a *whole organ,* the machinery of its vascular supply being included as well as its proper nervous components. So in this respect cerebral hemorrhage properly belongs where it is placed in Table VII, with the nervous system.

But the case is different from the embryological viewpoint. Suppose it be granted for the moment that there are specific differences between tissues originating from the different germ layers in respect of their likelihood to break down functionally under strain. Then clearly the tendency to any such specificity would be obscured if we charged to ectoderm the breakdown of any organ primarily originating from that germ layer, but where in fact the initial cause of the functional stopping of the proper ectodermic tissue was the prior breakdown of a part of the organ which was mesodermic in origin. This is precisely the condition of affairs relative to the pathology of cerebral hemorrhage.

Again, softening of the brain is really a necrosis of brain tissue resulting from a cutting off of its nourishment by stoppage of the circulation, which in turn may be due to arthritis, thrombosis, embolism or pressure. The same reasoning applies here as in the case of cerebral hemorrhage.

In so complicated a matter as the distribution of causes of death to their embryological base probably the most that can ever be hoped for, having regard to the enormous complications of structural development, is to get limiting values, within the range comprehended by which, the true fact may be reasonably supposed to lie. To this end Table XIII has been constructed. It with Table XII gives lower and upper limiting values for death rates chargeable to ectoderm and mesoderm. Table XIII is

made up in the same way as Table XII except that in the former items 64 and 65 are transferred from ectoderm to mesoderm. So far as I have been able to think the matter through it does not appear that the same complication may fairly be considered to arise in connection with the embryological assignment of any other "cause of death."

TABLE XII

Showing the Relative Influence of the Primary Germ Layers in Human Mortality

(Items 64 and 65 charged to ectoderm)

| Locality | Death Rate per 100,000 Due to Functional Breakdown of Organs Embryologically Developing from | | | | | |
|---|---|---|---|---|---|---|
| | Ectoderm | % | Mesoderm | % | Endoderm | % |
| U. S. Registration Area, 1906–10 | 191.1 | 14.3 | 425.2 | 31.8 | 719.6 | 53.9 |
| U. S. Registration Area, 1901–05 | 210.6 | 15.0 | 407.1 | 29.0 | 786.2 | 56.0 |
| England and Wales, 1914 | 177.1 | 14.4 | 374.0 | 30.3 | 681.5 | 55.3 |
| Sao Paulo, 1917 | 134.9 | 8.4 | 468.0 | 29.0 | 1,009.9 | 62.6 |

*C. Endoderm.*—The process of getting the figures here was to add together first the totals of Tables II and VI (respiratory system and alimentary tract) the organs represented being mainly endodermal in origin. Then there were *subtracted* from this total the following: (*a*) items 39 (cancer of the buccal cavity) and 0.34 of item 41 (cancer of the peritoneum, intestines, and rectum), leaving 0.66 of this latter item here for cancer of intestines; (*b*) items 117 (simple peritonitis) and 93 (pleurisy); (*c*) item 86 (diseases of the nasal fossæ). All of these items subtracted have been already placed with either ectoderm or mesoderm. Finally, there were *added* items 124 and 125 (diseases of the bladder and of the urethra) which were taken from the mesoderm for reasons already stated under that heading. Also there were added items 51 and 88 from Table IX (exophthalmic goiter and diseases of the thyroid body), because the thyroid arises from the epithelium lining the inner branchial furrows. The re-

sult finally obtained by the process described is that which appears in Tables XII and XIII under "endoderm."

TABLE XIII

SHOWING THE RELATIVE INFLUENCE OF THE PRIMARY GERM LAYERS IN HUMAN MORTALITY

(Items 64 and 65 charged to mesoderm)

| Locality | Death Rate per 100,000 Due to Functional Breakdown of Organs Embryologically Developing from | | | | | |
|---|---|---|---|---|---|---|
| | Ectoderm | % | Mesoderm | % | Endoderm | % |
| U. S. Registration Area, 1906–10 | 116.9 | 8.7 | 499.4 | 37.4 | 719.6 | 53.9 |
| U. S. Registration Area, 1901–05 | 137.3 | 9.8 | 480.4 | 34.2 | 786.2 | 56.0 |
| England and Wales, 1914 | 107.9 | 8.7 | 443.2 | 36.0 | 681.5 | 55.3 |
| Sao Paulo, 1917 | 101.3 | 6.3 | 501.6 | 31.1 | 1,009.9 | 62.6 |

The data of Tables XII and XIII are shown graphically in percentage form in Fig. 2.

The final results shown in Tables XII and XIII lead at once to a generalization of considerable interest and significance to the evolutionist. The figures show that in man, the highest product of organic evolution, about 57 per cent. of all the biologically classifiable deaths result from a breakdown and failure further to function of organs arising from the endoderm in their embryological development, while but from 8 per cent. to 13 per cent. can be regarded as a result of breakdown of organ systems arising from the ectoderm. The remaining 30 to 35 per cent. of the mortality results from failure of mesodermic organs. Taking a general view of comparative anatomy and embryology it is evident that in the evolutionary history through which man and the higher vertebrates have passed it is the ectoderm which has been most widely differentiated from its primitive condition, to the validity of which statement the central nervous system furnishes the most potent evidence. The endoderm has been least differentiated in the process of evolution, while the mesoderm occupies an intermediate position in this respect. An elaborate array of evidence might be presented on these points, but to do so would be

supererogation. It would amount simply to repeating any standard treatise on the comparative anatomy of the vertebrates, a branch of biological literature which one

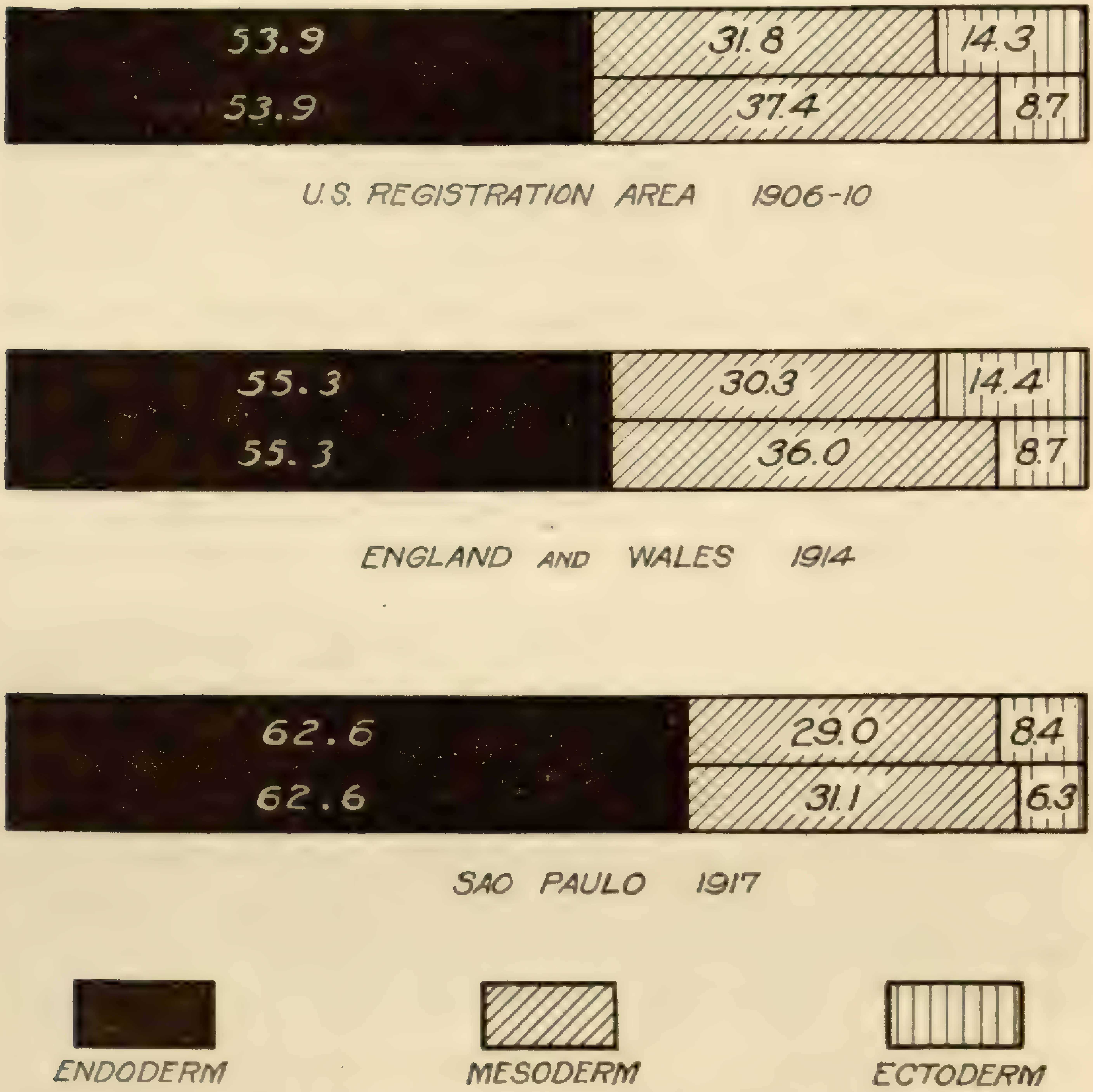


FIG. 2. Diagram showing the percentages of biologically classifiable human mortality resulting from breakdown of organs developing from the different germ layers. Upper bar of pair gives upper limit of mortality chargeable to ectoderm: lower bar gives lower limit of mortality chargeable to ectoderm.

may fairly assume that the readers of this paper are acquainted with, at least in general terms.

Degree of differentiation of organs in evolution implies degree of adaptation to environment. The writings of Darwin and Spencer, and in current times of Henry Fairfield Osborn, have demonstrated this point beyond question. From the present point of view we see that that germ layer, the endoderm, which has evolved or become differentiated least in the process of evolution is

least able to meet successfully the vicissitudes of the environment. The ectoderm has changed most in the course of evolution. The process of differentiation which has produced the central nervous system of man had as a concomitant the differentiation of a protective mechanism, the skull and vertebral column, which very well keeps the delicate and highly organized central nervous system away from direct contact with the environment. The skin exhibits many differentiations of a highly adaptive nature to resist environmental difficulties. It is then not surprising that the organ systems developed from the ectoderm break down and lead to death less frequently than any other.

The figures of Tables XII and XIII make it clear that man's greatest enemy is his own endoderm. Evolutionally speaking, it is a very old-fashioned and out-of-date ancestral relic, which causes him an infinity of trouble. Practically, all public health activities are directed towards overcoming the difficulties which arise because man carries about this antediluvian sort of endoderm. We endeavor to modify the environment, and soften its asperities down to the point where our own inefficient endodermal mechanism can cope with them, by such methods as preventing bacterial contamination of water, food and the like, warming the air we breathe, etc. But our ectoderm requires no such extensive amelioration of the environment. There are at most only a very few if any germs which can gain entrance to the body through the normal, healthy, unbroken skin. We do, to be sure, wear clothes. But it is at least a debatable question whether upon many parts of the earth's surface we should not be better off without them from the point of view of health.

These tables indicate further in another manner how important are the fundamental embryological factors in determining the mortality of man. Of the three localities compared, England and the United States may fairly be regarded as much more advanced in matters of public

health and sanitation than Sao Paulo. This fact is reflected with perfect precision and justice in the relative proportion of the death rates from endoderm and ectoderm. In the United States and England about 55 per cent. of the classifiable deaths are chargeable to endoderm and about 9 to 14.5 per cent. to ectoderm. In Sao Paulo 62.6 per cent. fall with the endoderm, and but 6.3 to 8.4 per cent. with the ectoderm. Since, as we have already shown, public health measures can and do affect practically only the death rate chargeable to endoderm this result which is actually obtained is precisely that which would be expected.

Finally, it seems to me that the results of this study add one more link to the already strong chain of evidence which indicates the highly important part played by innate constitutional biological factors as contrasted with environmental factors in the determination of the observed rates of human mortality. Here we have grouped human mortality into broad classes which rest upon a strictly biological basis. When this is done it is found that the proportionate subdivision of the mortality is strikingly similar in such widely dissimilar environments as the United States, England and Southern Brazil. It is inconceivable that such congruent results would appear if the environment were the predominant factor in human mortality. This conclusion does not overlook the fact that in some diseases the environment, in a broad sense, is unquestionably the factor of greatest importance. Nor does it imply that every effort should not be used to measure in every case the precise relative influence of constitution or heredity as compared with environment in the natural history of particular diseases. This constitutes one of the most pressing and difficult problems of medical science.

## VI

By way of summary it may be said that the purpose of this paper is to rearrange the rates of human mortality

as given in official reports of vital statistics, under the code known as the International Classification, into another classification upon a biological basis. The basis taken is organological, each "cause of death" is charged against that organ or organ system, the functional breakdown of which is fundamentally responsible for the death. It is found that from 85 to 90 per cent. of all statistically recognized causes of death can be subsumed under such a biological classification. It is found when this is done that the order of significance of the different organ systems in responsibility for human mortality is in general that of the following list, the arrangement being in descending order:

1. Respiratory system.
2. Alimentary tract and associated organs.
3. Circulatory system and blood.
4. Nervous system and sense organs.
5. Kidneys and related excretory organs.
6. Primary and secondary sex organs.
7. Skeletal and muscular system.
8. Skin.
9. Endocrinal system.

The arrangement differs slightly for different countries. If the further step is taken of referring the different organs and organ systems to the primary germ layers from which they embryologically developed, it is found that the death rates chargeable to organs of (*a*) ectodermic, (*b*) mesodermic and (*c*) endodermic origin stand to each other somewhere between the ratios of 1 to 2.3 to 4.4 and 1 to 4.4 to 7. The evolutionary and public health significance of these results is discussed at some length.

# ON THE REACTION OF TISSUES TOWARDS SYNGENESIO-HOMOIO- AND HETEROTOXINS, AND ON THE POWER OF TISSUES TO DISCERN BETWEEN DIFFERENT DEGREES OF FAMILY RELATIONSHIP

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In a series of papers our collaborators and ourselves have analyzed the action of tissues upon each other after transplantation carried out under varied conditions. We wish now to suggest certain general conclusions of wider significance which may be drawn from the facts which have been previously published by us or which will be published in the near future. No attempt will be made here to review the facts on which our conclusions are based and we must refer instead to our papers.[1]

In 1907 we first noticed that lymphocytes may appear around or in transplanted tissue. Subsequently we observed this occurrence in various tissues after transplantation. We thought it possible that the action of lymphocytes depended upon the relationship between host and transplant. This was confirmed by our experiments.

[1] Leo Loeb, *Archiv. f. Entwickelungsmech.*, 1897, VI, 1; 1907, XXIV, 638; 1911, XXXI, 456; 1898, VI, 297.

Leo Loeb and W. H. F. Addison, *Archiv. f. Entwickelungsmech.*, 1909, XXII, 73; 1911, XXXI, 44.

Llewellyn Sale, *Ibid.*, 1913, XXXVII, 248.

M. G. Seelig, *Ibid.*, 1913, XXXVII, 259.

Max W. Myer, *Ibid.*, 1913, XXXVIII, 1.

Cora Hesselberg, *Journ. Exp. Med.*, 1915, XXI, 164.

Cora Hesselberg, William Kerwin and Leo Loeb, *J. Med. Research*, 1918, XXXVIII, 17.

Leo Loeb, *Journ. Am. Med. Asso.*, 1915, LXIV, 726.

Leo Loeb, *Journ. Med. Research*, 1918, XXXVIII, 393; 1918, XXXIX, 189; 1918, XXXIX, 39; 1918, XXXIX, 71; 1918, XXXII, 353.

We found, however, that not only the lymphocytes, but also the blood and lymph vessels and fibroblasts behaved in a specific manner in accordance with the relationship between host and graft.

We assume that all the tissues of an individual have in common a certain chemical group which may be designated as individuality-differential. After transplanting a piece of an organ into a near relative of the donor of the tissue (syngenesiotransplantation), or into an unrelated individual of the same species (homoiotransplantation), or into an individual belonging to a different species (heterotransplantation), the individuality-differential is no longer adapted to its environment and acts as a syngenesio-, homoio-, heterodifferential, respectively. In this inadequate environment the individuality-differential assumes injurious properties, either directly or after interaction with the body fluids, some protein constituent of which contains likewise the individuality-differential or rather a group specifically combining with the individuality differential. It is probable that the second alternative represents the usual way in which the individuality-differential becomes a syngenesio-, homoio-, heterotoxin.

In certain cases the toxic character of the substance which originates after homoiotransplantation is strong enough to exert a direct injurious action on certain transplantation is strong enough to exert a direct injurious action on certain transplanted tissues, for instance, myxoid connective tissue and unstriated muscle. It can also directly interfere with those metabolic processes which lead to the production of epithelial pigment. In other cases, however, the toxic substances, while strong enough to modify the metabolism of the tissues, do not endanger the life of the transplant. This occurs after transplantation of glandular structures like kidney or thyroid or epithelial structures in general. But secondarily this change in the metabolism of the transplanted tissue alters the reaction of the host cells towards the graft. The lymphocytes are attracted, the vascular supply is dimin-

ished; fibroblasts are given freer access to the transplant, and moreover, the fibroblasts undergo secondary changes; they form fibrous tissue, while after autotransplantation the cytoplasm usually remains to a much greater extent intact without undergoing the secondary changes in contact with the grafted cells.

These toxic substances not only interfere with those proliferative processes which are of a regenerative character in the more restricted sense, but also with others caused by growth substances; thus they interfere with the production of experimental deciduomata in the uterus or with compensatory hypertrophy of the thyroid after homoiotransplantation, without however necessarily preventing these growth processes entirely. Ultimately they endanger the life of the exposed tissues, usually indirectly through the action of the lymphocytes and connective tissue cells, more rarely directly.

Furthermore the syngenesio-, homoio- and hetero-differentials may act secondarily as antigens directly or after interaction with the body fluids and then call forth the production of immune substances. Such immune substances however in most cases become demonstrable only if the strangeness of the antigen used has been very marked; this is for instance the case if a hetero-differential serves as an antigen (Schoene, M. S. Fleisher). A homoio-differential is only in some cases able to become an antigen. Under certain conditions homoio-haemolysins can appear (Ehrlich and Morgenroth) or haemagglutinins (von Dungern and Hirschfeld) and furthermore homoio-immune substances can be produced against growing tumors and embryonal tissues (Fichera, Peyton Rous). In the case of tumor immunity lymphocytes play a very prominent part. This has been noted by a number of investigators. More recently the significance of lymphocytes in immunity has been demonstrated in varied experiments especially by J. B. Murphy and his collaborators. However, as we shall see presently, the direct reaction of tissues (including lymphocytes) against syngenesio-, homoio- and heterotoxins is a finer bio-

chemical reaction than any reaction known so far and more delicate than the immune reactions.

These substances which function as individuality-differentials are produced in the active metabolism of cells; they can be demonstrated through their reaction after transplantation of metabolically active tissue, but not after transplantation of erythrocytes contained in a blood clot. The syngenesiotoxins are least toxic. Usually they call forth a lymphocytic reaction only slowly; but ultimately the lymphocytes appear even here and invade and destroy the strange tissue. At a time when the vascular and fibroblastic reactions should occur, the toxins may as yet be so weak that these two kinds of tissues may behave normally. In other cases, however, even the syngenesiotoxins may be strong enough to call forth an altered reaction of vessels and fibroblasts; the vessels may fail to be present in a large number by the graft and the fibroblasts may produce more fibrous tissue. This occurs typically in response to homoiotoxins; furthermore, in the presence of homoiotoxins lymphocytes appear early and in large masses. They, as well as the fibroblasts, may invade and destroy the strange tissue. In the case of the heterotoxins the direct injurious effect of the body fluids is much more pronounced, and in a general way this effect is the greater, the more distant the relationship between host and graft, but in this case also the cellular reactions of the host may secondarily contribute to the destruction of the strange tissues. The fibroblasts of the host have a tendency to form dense fibrous tissue around the graft and the blood and lymph vessels are usually extremely sparse in the direct neighborhood and in the interior of a heterograft; apparently the strange cells do not exert an attracting or stimulating influence on the growth of capillaries. While thus in these two respects the heterotoxins produce an effect which might have been foreseen from a study of homoiotoxins, the behavior of the lymphocytes seems to be different from what might have been expected. Instead of being attacked by lymphocytes even more actively

than the homoio-tissues, the lymphocytic reaction is on the contrary decidedly weaker in the case of tissues of other species. Lymphocytes do not usually invade and destroy the tissue of a foreign species, although they may collect in a considerable number in the neighborhood of the graft and may occasionally invade it here and there in small areas and destroy these. They may appear in large quantities after the graft has been destroyed. This relative inactivity of the lymphocytes is surprising, if we consider the much greater strangeness between graft and host which results from heterotransplantation. But it may be just on account of the great strength of the heterotoxins that the reaction is weakened. We have seen above that the development of the specifically attracting substances depends upon the active metabolism of the graft as evidenced by the failure of the erythrocytes to attract the lymphocytes. In the case of heterotransplantation the action of the toxins may be so strong that it interferes with these specific metabolic activities of the transplanted cells, and thus the reaction would naturally become weaker. It may also be that the scarcity of vessels around and in the graft may contribute to this result, these vessels acting as the channels through which the lymphocytes reach the graft. But that this factor can not be the only reason is clear from a study of certain areas of the transplant occasionally found in which the blood vessel supply may be somewhat better, but in which, nevertheless, the lymphocytic infiltration of the graft is absent or slight.

There is then a graded reaction of various tissues of the host towards transplants. At one end of the series we find the autotransplants; these are followed by the various kinds of syngenesio-, by homoio- and heterotransplants. Autotransplants call forth a marked vascular reaction. This reaction decreases in the direction towards heterotoxins; it is already very weak in most cases of homoiotoxins. The fibroblasts remain relatively well preserved, form least fibrillar material and tend to the production of myxoid tissue under the influence of

those conditions which prevail in the case of autotransplantation. Here they are prevented at most places from breaking into the glandular structures; in the presence of homoio- and hetero- and even of syngenesio-toxins they tend in a higher degree towards the production of fibrillæ. Lymphocytes are attracted by syngenesiotoxins and still more by homoiotoxins; here a maximum is reached. A decrease occurs again in the region of the heterotoxins. The number of fibroblasts invading the glandular transplant is likewise greatest in the case of homoiotransplantation. Again a decrease seems to occur in the case of heterotransplants.

We may express these facts also in a somewhat different way. Under conditions of autotransplantation the tissues give off substances which correspond to the identity of the individuality-differentials in the host and graft. These substances, which may be designated as "auto-substances," stimulate directly or indirectly the vascular supply of the tissues belonging to the same individual. And they also exert directly or indirectly a definite effect upon the fibroblasts, keeping them active and preventing them from undergoing those processes which probably represent a decline in normal metabolism and which lead to the production of fibrous tissue at the expense of the healthy active cytoplasm. In the absence of these auto-substances, the vascular supply is lessened in direct proportion to the greater distance of the relationship between host and graft. To a certain extent syngenesiotoxins still take the place of the auto-substances characteristic of the interaction between the own tissues and body fluids. Some syngenesiotoxins however can do so only to a very slight extent; homoiotoxins are still less able to take the place of auto-substances and least of all heterotoxins. In the case of homoio- and heterodifferentials the stimulating effect of the "auto substances" is lacking; they behave in certain respects like foreign bodies.

On the other hand the autosubstances exert no attracting influence on the lymphocytes, while the syngenesiotoxins

exert at first a very slight, but gradually cumulative, effect; the homoiotoxins exert the maximum effect; and then a decrease again takes place in the region of the heterodifferentials.

It might be suggested that the same auto-substances play a part in the interaction between the normal tissues of an individual. It is quite evident that the effect of the autosubstances would be beneficial to the tissues and contribute to their nourishment. Thus the body would possess a system of very finely acting chemical autoregulators which keep the vascular supply at the point of greatest intensity and prevent the fibroblasts from forming dense fibrous tissue which again would interfere with the nourishment of the tissues and which insure such an interaction of tissues that the normal structure results. Strange tissues do not exhibit the effect of such autosubstances, even if such strange tissues should temporarily multiply mitotically, and therefore they perish in the end.

On the other hand, autosubstances are indifferent, as far as the lymphocytes are concerned, while syngenesio- and homoio- and to some extent heterotoxins attract them. The lymphocytes as well as invading fibroblasts, are a destructive element and the effect of both is eliminated through the action of autosubstances; again a beneficial adaptation. Hypothetically we might thus explain in part at least the changes of tissues in old age. The effect of the autosubstances would be strongest in young individuals and decrease in the less actively metabolizing older tissues; and here we find, therefore, changes which are characteristic of a decrease in the autosubstances, or rather of specific metabolic substances which have the "auto" character and at the same time are characteristic of the specific organ activity. Therefore in old age the fibroblasts around the parenchyma from fibrous tissue and the vascular supply decreases. In the embryo we find least fibrous and most myxoid tissue and the best vascular supply.

Correspondingly we find least dense fibrous tissue around actively metabolizing parenchyma, while around the metabolically less active excretory ducts there is usually a coat of much more dense fibrous tissue. Thus it might be tentatively suggested that a decrease in these autosubstances, to the existence of which the results of tissue transplantation point, is at least partially responsible for the changes which take place in old age. Other factors of a different nature play certainly a part. Thus the corpus luteum stimulates the activity of the uterine connective tissue and epithelium. In later periods of life this stimulating influence is lacking.

While the autosubstances increase directly or indirectly the vascular supply, they very effectively limit the growth of fibroblasts in contact with epithelial elements. We have previously shown through transplantation experiments that during regeneration the epithelium limits the growth of the connective tissue.[2] In a similar way perhaps the endothelium of the blood vessels limits the growth of the connective tissue. Our further experiments lead to the conclusion that this restraining influence exerted by endothelium and epithelium upon connective tissue migration and proliferation is a specific effect. The auto-substances possess it in the highest degree. It is less marked in the case of syngenesio- and still less in the case of homoiotoxins. But even the hetero-substances still exert a certain restraining influence, at least temporarily on the connective tissue and they are thus in this respect more effective than foreign bodies. It is again clear that considering the tendency of fibroblasts and lymphocytes to migrate actively into parenchyma and to destroy it, the epithelial structures can be assured of preservation only, if the auto-substances possess some kind of a restraining influence on the connective tissue.

It is probable that not only in old age but also under certain pathological conditions, the activity of these auto-

[2] Leo Loeb, *Arch. f. Entwickelungsmech.*, 1898, VI, 297 (p. 319).

substances is interfered with, and that thus cirrhotic processes may be produced in various parenchymatous organs. The usual assumption is that all these fibrous changes are the result of an actual destruction of the epithelium, that they are therefore due to regeneration in the more restricted sense of the term. It is probable that both these processes may occur in different cases.

As we have shown, various tissues, but particularly the lymphocytes, are able to distinguish not only between tissues belonging to the same and other individuals of the same species, but even to recognize the difference in degree of relationship. They behave in a graded manner towards their own tissues, the tissues of a brother, mother, the tissues of a not related individual of the same species and the tissues of an individual of a different species. These reactions must be in response to specific substances given off by these tissues, the autosubstances, the syngenesio-, homoio- and heterotoxins. Whether these substances are identical with the individuality-differentials residing in the tissues or whether they are due to an interaction between the individuality-differentials residing in the tissues and adapted substances in the body fluids can not be decided definitely at present; however, the latter assumption seems to be more probable. These responses represent to our knowledge the finest biochemical reactions which are known at the present time and surpass even the immune reactions which permit us to distinguish only between different species and in a few cases only between individuals of the same species.[3]

These reactions we may hope will contribute to an understanding of the behavior and the functions of tissues in general as distinct from the specific functions of organs which under certain conditions are added to

[3] It is possible that a further development of the immunological methods used by von Dungern and Hirschfeld and by Todd and White (*Proc. Royal Soc.*, 1910, LXXXII, 416) will also lead to a differentiation in the relationship of individuals of the same species.

these more general tissue reactions, and thus they may contribute to a physiology of tissues as contrasted with the physiology of organs. Further experiments will have to determine, how far the effect of the parenchyma on the fibroblasts and blood vessels is a direct one and how far secondary interactions between fibroblasts and blood vessels enter into these reactions.

These various substances (autosubstances, syngenesio-, homoio- heterodifferentials) have in addition to the functions which concern the effect of cells upon each other added functions of a specific nature. This is indicated by the existence of specifically adapted substances like the tissue coagulins which determine in a specifically adapted manner the coagulation of the blood of various species.[4]

[4] Leo Loeb, *Montreal Med. Journal,* July, 1903; *Virchow Archiv,* 1904, CLXXVI, 10; *Biochem. Zeitschrift,* 1910, XXVIII, 169.

# THE INDIVIDUALITY-DIFFERENTIAL AND ITS MODE OF INHERITANCE

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In a preceding communication we have shown that all the tissues of an individual have in common a chemical characteristic through which they differ from other individuals of the same species. This characteristic may be designated as the individuality-differential. It is probable that in the circulating body fluids these individuality-differentials or substances specifically adapted to them are likewise present. The interaction of cells and substances which possess the same individuality differential leads to the production of autosubstances which are responsible for various conditions of tissues. But if, through transplantation, the individuality-differentials become converted into syngenesio-, homoio- or hetero-differentials toxic substances are produced, the syngenesio-, homoio- or heterotoxins which lead to tissue reactions of different kinds as we have described in the preceding communication.

In the process of fertilization usually two homoio-differentials combine to form a new individual. Through transplantation of tissue it is possible to determine whether the individuality-differential of the child is identical with the individuality-differential of one of the two parents or whether its character is intermediate. If the inheritance of the individuality-differential should behave like a simple Mendelian monohybrid character, all the offspring of the first generation would have the same individuality-differential and the individuality-differential of one of the two parents would probably dominate.

Interchange of tissues between the children (brothers and sisters) should give results identical with those of autotransplantation, and transplantation of tissues from one of the two parents to a child should in all children give the same result, and the results should be those of either auto- or homoiotransplantation. Or, it might be possible that in the offspring a blending of the individuality-differentials of both parents occurs. It might furthermore be possible that in all children the same kind of blending occurred or that all intermediate degrees of blending of the differentials of father and mother be found.

In using transplantation of tissue as a means of determining which of these possibilities is realized, we have to take into account the difference in the situation of host and graft. Under the usual conditions of transplantation the host is a selfsufficient organism and is not in any essential manner dependent for his nourishment upon the graft. The graft on the contrary depends upon the host for its nourishment. The relation between host and graft is therefore not that of simple reciprocity. This relation may be important in interpreting certain results of transplantation as we shall see later.

We have carried out two series of experiments in which we analyzed the mode of inheritance of the individuality-differential, one in the rat[1] and a second one in the guinea pig.[2] In the former we transplanted simultaneously pieces from different organs into rats; in the second we used the thyroid gland for transplantation. We transplanted tissues from parents to children, from children to mother and from brothers to brothers. Both series, in the rat and guinea pig, gave the same result as far as the main problem is concerned; the individuality-differentials of the children are intermediate between those of the two parents; but all kinds of intermediate conditions

[1] Leo Loeb, *Journ. Med. Research*, 1918, XXXVIII, 393 (here the literature is discussed).

[2] Leo Loeb, *Journ. Med. Research*, 1918, XXXIX, 39.

are found varying between those approaching identity of individuality-differentials on the one extreme and homoio-differentials on the other.

In the guinea pig we analyzed further the difference in the results after transplantation of tissues from brother to brother, from mother to child and from child to mother. We found transplantation from brother to brother to give the best results, but even here the mixing of the individuality-differentials called forth the development of toxins, syngenesiotoxins, which usually were relatively mild, but in certain cases would be more severe. Transplantation from child to mother led to the production of toxic effects which were almost as marked as those produced by the homoio-toxins. Transplantation of tissues from mother to child on the whole resembled that of transplantation from brother to brother, but seemed to be somewhat less favorable. In the rat there were likewise indications that the transplantation from child to mother was more unfavorable than the others, but a decided difference between transplantations from mother to children and from brother to brother could so far not be established. However, the indicator of effects which we used in our guinea pig series was finer than that in our rat series.

We have begun experiments to determine the behavior of individuality-differentials in the second generation. It seems that here too the results are intermediate, but further experiments need to be carried out, before a definite statement can be made.

In a provisional way we may attempt to explain these results as follows. In most cases each individual has at least two sets of individuality-differentials, one inherited from the father, the other from the mother. Each set again consists of two kinds of differentials. There may be added individuality-differentials from some further distant ancestors, but this complication may be ignored at present; in certain individuals the differentials of either father or mother are lacking. We may assume

that several or all of the chromosomes of the father are characterized by certain chemical groups which would be the same in the cells of the same individual, but would differ in the cells of different individuals. Each cell of the child obtains a combination of chromosomes which is the same in the same individual, but differs in the case of different brothers or sisters. The chemical individuality-character of the chromosomes should lead to analogous chemical differences consisting perhaps in the formation of chemical sidechains attached to proteins; they should be present primarily in cell proteins and secondarily in the proteins of the body fluids. While the individuality-differentials in the tissues exist perhaps even in the embryo, there are some indications that the adapted substances in the body fluids originate after birth. These side chains must be identical in all the proteins of the same individual and differ in the case of different individuals. We should then expect that the individuality-differentials of the children, being a mixture of those of the parents, however in proportions which differ in the case of different children, should be intermediate between those of the parents. Extremes in the children may be almost identical with one or the other parent. In transplanting tissue from one brother to another the graft would in most cases find in the host the same characteristic groups which its own cells possess, but in a somewhat different quantitative relationship. Therefore the life of the graft which finds all the characteristic substances could be sustained; but the quantitative differences which exist in most cases would gradually lead to toxic effects which ultimately endanger the life of the graft. In some cases, however, the host would lack altogether some of the chromosomes or groups present in the brother and then the result would be more unfavorable, somewhat approaching that of homoiotransplantation.

In the case of transplantation from child to mother on the other hand the graft would lack one half the chromo-

somes and therefore the corresponding chemical groups present in the cells of the graft. The result should therefore approach that of homoio-transplantation, which we indeed find to be the case. After transplantation from mother to child the graft finds in the host in many cases the chemical groups it possesses itself, but again the proportion of chemical groups in host and graft (corresponding to that of the chromosomes) differs here more than in the case of two brothers and these quantitative differences might lead to a greater incompatibility between graft and host than in the case of transplantation from brother to brother. On this assumption we might furthermore expect that in certain rare cases even homoio-differentials should show a similar constitution and might therefore permit a successful transplantation into a not related individual.

While the somatic tissues require for their normal life identity of individuality differentials with which they come into contact, the germ cells on the contrary are normally adapted to contact with homoio-differentials in the chromosomes and as T. H. Morgan[3] has shown in Ciona secondary mechanisms may even make auto fertilization impossible.

In man, Landsteiner, Moss and others found a peculiar distribution of isoagglutinins into three or four groups, which are apparently independent of the parentage of the individuals concerned. Such a condition seems to be peculiar to man and has not been found in animals (Hecktoen).[4] In certain animals, however, von Dungern[5] and Hirschfeld succeeded through immunization to demonstrate the existence of two kinds of isohemagglutinins and of the corresponding antigens and thus of four classes of individuals. As our transplantations show conditions in the tissues cannot be the same as in the red blood corpuscles, if we should judge the constitution of

[3] T. H. Morgan, *Biological Bulletin*, 1905, VIII, 313.

[4] L. Hecktoen, *J. Infect. Diseases*, 1907, IV, 297.

[5] V. Dungern, *Munch. Med. Wok.*, 1910, Vol. 57, p. 293, p. 740.

the latter on the basis of these agglutination tests. In the case of the tissues we have to assume the existence of individuality-differentials which are composed of multiple chemical groups; therefore Mendelian heredity would be that of multiple factors. It is not improbable that even in the case of tissues the number of these groups is limited and that all the individuals of the same species have a choice only between a relatively small number of groups which is characteristic of each species and that the different individuals of a species differ from each other through the combination of these groups which each individual possesses. Other chemical groups would be characteristic of species and in this case also the number of groups which constitute a species differential may be limited.

The explanation for the facts of inheritance of the individuality-differentials which we attempted in this note is regarded by us at present as of an entirely provisional character. So far, however, it seems to agree with the facts as they are known; but we have no doubt that as investigations progress still further it may require certain, perhaps fargoing, modifications. It finds, however, support in the investigations of Landsteiner,[6] Pick and Obermeyer and others who have shown that the immune reaction-specificity of protein substances can be experimentally altered through changes in chemical side chains which are added to these proteins.

6 Karl Landsteiner u. Hans Lampl, *Biochem. Zeitsch.*, 1918, LXXXVI, 342.

# LINKAGE IN RATS[1]

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AT the present time there are at least five factor pairs or series known in rats. Some of these have been described only quite recently, and it may therefore be profitable to tabulate all of them and to mention some of their interactions.

These genes are:

1. *R*, black-eyed; *r*, red-eyed.
2. *P*, black-eyed; *p*, pink-eyed.
3. *S*, self; $S_i$, Irish; $S_h$, hooded.
4. *A*, agouti; *a*, non-agouti.
5. *C*, intense pigmentation; $C_r$, non-yellow; $C_a$, albinism.

Color varieties homozygous for one or the other of the first two recessive genes mentioned above were described by Castle (1914). Both are yellow-coated. A *PPrr* animal is yellow with eyes of a reddish tint, while animals of the composition *ppRR* or *pprr* are yellow with pink eyes. Pink-eyed yellows, therefore, may be of two kinds, those carrying *R* and those lacking it. Animals carrying both *P* and *R* are black-eyed and have black coats. Pink-eyed yellows, carrying *R* (*ppRR*), mated to red-eyed (*PPrr*) produce *PpRr*, black-eyed animals having black coats. Pink-eyed yellows lacking *R*, and therefore of the composition *pprr*, when mated to red-eyed yellows (*PPrr*) give *Pp rr*, or red-eyed yellows.

Castle and Wright (1915) and Castle (1916) present evidence that the genes above described are linked. When pink-eyed yellows (*ppRR*) were crossed with red-eyed

[1] Papers from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin, No. 21. Published with the approval of the Director of the Station.

yellows (*PPrr*), the *p* and *R* of the pink-eyed and the *P* and *r* of the red-eyed tended to be linked, the crossing over being about 17 or 18 per cent.

More recently Castle (1916) has given evidence indicating that Irish pattern ($S_i$), entirely pigmented except for a white patch on the belly, is allelomorphic to both self ($S$) and the hooded pattern ($S_h$). Whiting and King (1918) have shown that the non-yellow condition ($C_r$) corresponds to that found in guinea-pigs and is similarly allelomorphic to both complete pigmentation ($C$) and albinism ($C_a$). Dilute pigmentation ($C_d$) has not as yet been found in rats. Castle (1916) presents experimental proof that the allelomorphs of pink-eyed (*p*) and red-eyed (*r*), or *P* and *R*, are linked with albinism, $C_a$. This would put the three genes on the same chromosome. Castle's data are too few for the exact degree of linkage to be determined from them.

Up to the present no results have been published of a direct attempt to determine the possible linkage relations of self ($S$) and agouti ($A$) to each other or to the other three linked genes. Data bearing on this point will be presented in this paper. It will also be shown that so far as our evidence goes the genes $R$ and $C_a$ are absolutely linked.

The first crosses made were for the purpose of determining the linkage relations of the red-eyed pair of allelomorphs ($R$ and $r$) with the self series ($S$, $S_i$ and $S_h$). Red-eyed selfs ($rSPa$) were crossed with black-eyed hooded ($RS_hPa$) and the resulting animals were therefore self[2]-blacks ($rS \cdot RS_h$).[3] These self-blacks were then bred back to the double recessives, red-eyed hooded ($rrS_hS_h$). The offspring from this mating are shown in Table I. From this it will be seen that red-eyed and self are entirely independent in heredity.

[2] With my animals every heterozygous self ($SS_h$) had a white patch on the belly.

[3] *P* and *a* may be disregarded since both parents were alike with respect to these genes.

TABLE I

$rS \cdot RS_h \times rrS_hS_h$

| Non-crossovers | | Crossovers | |
|---|---|---|---|
| Black-hooded, $RS_h$ | Self-yellow, $rS$ | Self-black, $RS$ | Yellow-hooded, $rS_h$ |
| 49 | 42 | 49 | 48 |

Later the opportunity was offered for studying the relation of agouti ($A$) to the above two genes. A red-eyed self carrying agouti[4] ($rSA$) was mated to black-eyed hooded blacks ($RS_ha$) and the resulting offspring, self agoutis ($rSA \cdot RS_ha$), were bred back to the triple recessives, red-eyed hooded ($rrS_hS_haa$). From this mating were obtained the animals shown in Table II. In this case also there is no indication of linkage since all of the classes are of approximately the same size.

TABLE II

$rSA \cdot RS_ha \times rrS_hS_haa$

| $ARS$ Black-eyed Self Agouti | $ARS_h$ Black-eyed Agouti Hooded | $ArS$ Red-eyed Self-red | $ArS_h$ Red-eyed Red-hooded | $aRS$ Black-eyed Self-black | $aRS_h$ Black-eyed Black-hooded | $arS$ Red-eyed Self-yellow | $arS_h$ Red-eyed Yellow-hooded | Total |
|---|---|---|---|---|---|---|---|---|
| 25 | 29 | 23 | 21 | 23 | 24 | 25 | 26 | 196 |

If we disregard the agouti gene in the above cross, the results from Table II may be combined with those from Table I. This has been done and is given in Table III. The combined results merely give added proof to the deductions drawn from Table I, that no linkage has occurred.

TABLE III

TABLES I AND II COMBINED

$rS \cdot RS_h \times rrS_hS_h$

| Non-crossovers | | Crossovers | |
|---|---|---|---|
| $RS_h$ | $rS$ | $RS$ | $rS_h$ |
| 102 | 100 | 97 | 95 |

[4] Red-eyed animals carrying agouti are of a decidedly deeper red than those lacking it (which seem dilute in comparison) and are easily distinguishable from the latter.

We may therefore conclude that the known genes in rats arrange themselves into three groups. Into one may be placed agouti ($A$) and its allelomorph, non-agouti ($a$); into the second, self ($S$), with its allelomorphs, Irish ($S_i$) and hooded ($S_h$); and into the third the three genes $R$, $P$ and $C$ with their allelomorphs.

As before stated, Castle showed that $R$ and $P$ were linked to albinism ($C_a$), but he did not determine the exact degree of their linkage.[5] Evidence on this point concerning the linkage of $R$ and $C_a$ will be presented in the following pages.

Two of the heterozygous self agoutis ($rSA \cdot RS_ha$) used in determining the linkage relations of $R$, $S$ and $A$, when mated together produced some albino offspring. The father of the self agoutis, ♂ 105A.1, a red-eyed self, carrying agouti ($rrSSAA$), could not have carried albinism himself since he had 17 non-albino offspring when mated back to one of his daughters, ♀ 126B.4, known to be carrying the albino gene. The mother of the self agoutis, ♀ 98A.2, a black-eyed black hooded, must therefore have been heterozygous for albinism, and accordingly would be of the composition $RRS_hS_haaCC_a$. Her offspring that gave birth to albinos would of necessity have the zygotic formula $RrSS_hAaCC_a$. These when inbred should have had albino offspring of various compositions, if there had been no linkage. For every albino that was homozygous for a dominant gene we should have expected two that were heterozygous and one that was homozygous for the recessive.

Since there were six albinos born from the agouti × agouti mating, it seemed quite probable that one at least would be found that was recessive for all the genes. Ani-

[5] In May, 1919, the author was informed by Professor Castle that he had conclusive evidence that $R$ and $C_a$ were completely linked. At that time he had obtained about 200 non-cross-over and no crossover gametes. While this paper was going through the press Castle's report (1919) on linkage in rats has appeared as Publication No. 288 of the Carnegie Institution or Washington. In studying the linkage relations of albinism and red-eyed he obtained 433 non-cross-over gametes and one somewhat doubtful crossover.

mals of this sort could be used for further linkage work. Even if none were found it seemed very probable that from them albinos of this description could be produced.

Accordingly all six albinos were mated to red-eyed yellow-hooded animals ($rrS_hS_haaCC$) in order to determine their compositions. The zygotic formulæ of the six proved to be as follows:

$$1RRS_hS_hAA,$$
$$2RRS_hS_hAa,$$
$$1RRSS_haa,$$
$$1RRS_hS_haa,$$
$$1RR.$$[6]

The one outstanding feature was that all six albinos were homozygous for black-eyed ($R$), although without linkage one should have expected that at least one would be red-eyed ($rr$) and several heterozygous ($Rr$). This gave a very strong indication of linkage and so further work was pursued with this in view.

To simplify matters we shall disregard all the genes but $R$ (and its allelomorph $r$) and $C$ with its allelomorph $C_a$, and we shall also assume complete linkage. When the above six albinos ($RRC_aC_a$), therefore, were crossed with red-eyed yellows ($rrCC$) their black-eyed offspring would be of the composition $RC_a \cdot rC$. These black-eyed animals when inbred, assuming complete linkage, would behave as follows:

$$RC_a \cdot rC \times RC_a \cdot rC = 1\,RC_a \cdot RC_a \text{ (albino)} : 2\,RC_a \cdot rC \text{ (black-eyed blacks)} : 1\,rC \cdot rC \text{ (red-eyed yellow)}.$$

[6] Black-eyed ($R$) animals may be distinguished from red-eyed ($r$) or albinos ($C_a$) at birth by the fact that their eyes already appear dark through the skin, while red and albino eyes are as yet devoid of pigment. It is only about two weeks after birth or just before the eyes open that the red eyes have enough pigment in them so that they can be distinguished from albinos or pink-eyed ($p$). One of the albino females being tested had 11 black-eyed ($R$) young which through some oversight were disposed of before their other characters were recorded.

The albinos from this mating when mated to their red-eyed yellow litter mates, or to red-eyed yellows from similar matings, should give nothing but black-eyed blacks ($RC_a \cdot rC$). This would prove for both albinos and red-eyed yellows that each was the result of the union of two non-crossover gametes. The black-eyed blacks when mated together should behave just like their black-eyed black parents, *i.e.*, produce all three phenotypes. In this case also this would prove that two non-crossover gametes had united in the production of each black-eyed black parent.

The above policy has now been pursued for some time. From the mating together of $RC_a \cdot rC$ black-eyed blacks the following offspring have resulted:

23 albinos, 57 black-eyed blacks, 31 red-eyed yellows.

For some reason there is a deficiency of albinos. As previously explained, black-eyed blacks may be distinguished at birth from either albinos or red-eyed yellows by the color of their eyes. In the above cross five animals died early, but none of them were black-eyed. They died before one could be sure whether they were albinos or red-eyed yellows. If there was selective mortality, and they were all albinos, the deficiency of albinos would thus be explained.

So far 13 albinos from $RC_a \cdot rC \times RC_a \cdot rC$ matings have been tested and all proved to be $RC_a \cdot RC_a$; therefore they are the result of the union of 26 non-crossover gametes. Similarly, 12 black-eyed blacks proved to be $RC_a \cdot rC$ and 10 red-eyed yellows, $rC \cdot rC$. The total number of non-crossover gametes going into the production of the above animals is 70, and is at least a fair indication that $R$ and $C_a$ are completely linked. More results are necessary in order to make this conclusive. Since the amount of crossing-over between $R$ and $P$ is already known (17–18 per cent.), and since $R$ and $C_a$ are completely linked, we should expect to find the same percentage of crossing-over occurring between $P$ and $C_a$ as between $P$ and $R$.

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# SOME HABITAT RESPONSES OF THE LARGE WATER-STRIDER, GERRIS REMIGIS SAY. III

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## VI. Discussion of Experiments at White Heath

*1. Rôle Played by Vision.*—In regard to the experiments at White Heath there is little to be said more than already has been stated in the discussion in connection with the responses of the water-striders, during severe drought, in the dry bed of the stream. In general, the majority of the gerrids found their way back to the brook, when removed from it to distances of one, two, three, and four yards. This was true whether they faced the brook, were placed with their bodies parallel to its banks, or faced directly away from the water. Water-striders with their heads turned away from the stream took a little longer time to reach the brook than was true of the other hemipterons, and they also evinced more random movements. Occasionally a gerrid wandered astray and seemed unable to reach the brook.

At such short distances away from the water, as have been mentioned, it is very probable that the hemipterons find their way back to it mainly through the sense of sight. It is a well-known fact that many species of aquatic Hemiptera respond positively to light as a stimulus, indicating that vision must play an important part in the behavior of the members of this group. Among these are the water-striders *Gerris orba* (Essenberg, 1915, p. 400), *Gerris remigis*, *Gerris marginatus* (Riley, MS.), and probably *Gerris thoracicus, Gerris tristan* (Kirkaldy, 1899, p. 110). Not only do certain water bugs respond positively to light, but Cole (1907, p. 387) has proved the truly remarkable fact that *Ranatra fusca* possesses the ability

to discriminate between two luminous areas of different size, even though they are of the same intensity. Water-striders may possess this sort of discrimination.

At this point I desire to direct attention to certain interesting experiments of Parker (1903) on the butterfly, *Vanessa antiopa* Linnæus, because of their probable bearing on some of the responses of *Gerris remigis*. According to this writer (1903, p. 467),

> *Vanessa antiopa* . . . [is able to] discriminate between light derived from a large luminous area and that from a small one, even when light from these two sources is of equal intensity as it falls on the animal. These butterflies usually fly toward the larger areas of light.

He (1903, p. 465) remarks that in the sunlit spots in the woods, this butterfly responds to the large areas of sunlight rather than to the smaller ones. This form of response applies also to the sun, although

> the retinal image of the sun must be vastly brighter than those of all other spots [of sunlight].

Furthermore, writing of the way in which *Vanessa antiopa* finds a patch of sunlight, he (1903, p. 464) makes the following statement:

> This patch [of sunlight] is found not through the accidental wandering of the butterfly into it, but by the butterfly's taking a direct course to it, precisely as the insect finds a single light window in an otherwise dark room. The directive influence, then, is not the intense sunlight that makes the patch, but the much less intense reflected light radiating from the patch. This must form a localized spot on each retina of the butterfly, and it is the position of these spots that determines the direction of flight.

The surface of the water in a brook forms an excellent reflecting surface, either for moonlight or for sunlight, and it is probable that the gerrids respond to such reflected light much in the same way that they do to artificial light. Or they may respond to water, or rather to the reflections from its surface, according to the same general principle that *Vanessa antiopa* responds to patches of sunlight. These areas of sunlighted water in the brook must have much the same appearance to insects with image-forming eyes, such as water-striders, as do

the sunlit areas in the woods. The gerrids go toward the water—not quite so directly as *Vanessa antiopa* moves toward areas of sunlight—with but few preliminary random movements, except in the case of those having their heads directed away from the brook, in much the same fashion and probably for much the same reason that *Vanessa antiopa* goes toward sunlit spots in the woods.

I observed, as early as the summer of 1911, that these water-striders respond to moving objects and shadows more promptly than they do to stationary ones. In the early fall of 1918, I discovered that individuals of *Gerris remigis,* confined in an aquarium, respond definitely and in a pronounced manner to a moving incandescent electric light and also to frequent changes in the position of such a light. Essenberg (1915, p. 402) states that in *Gerris orba*

> The sense of sight is keenly developed, the insects detecting a moving object or a shadow very quickly.

The responses of *Ranatra fusca* to a moving light and also to a light frequently changed as to position are well known through the admirable work of Holmes (1905). He (1907, pp. 160, 161) has also pointed out that the young of *Ranatra quadridentata* respond to changes in position of a light. Therefore, a brook with a current of moderate velocity is more likely to be seen by the gerrids than is still water. The riffles and small waves serve as additional reflecting surfaces for diffuse daylight and sunlight, which facts aid in making the position of the brook still more noticeable to the water-striders.

2. *Rôle Played by Moisture.*—It is possible that moisture from the brook diffusing through the atmosphere may serve, to a certain extent, as a stimulus which may produce a positive response when the water-striders are in such close proximity to the stream as previously has been indicated. However, I am much in doubt of such an explanation. The plan to prevent the gerrids from seeing

the water, or perhaps the reflections from it, and yet not to obstruct the diffusion of moisture from the brook did not produce results sufficiently definite from which to draw conclusions. There is much doubt as to the rôle played by moisture in influencing water-striders to move toward the brook.

### VII. Discussion of Experiments at Syracuse

*1. Rôle of Vision.*—The experiments conducted near the large pool in the brook at Syracuse added little information to what has been stated concerning the work near White Heath. I believe that vision was the main factor in assisting the gerrids to reach the large pool of water. There were no obstacles to obstruct the view of the gerrids, the surface of the ground being smooth and flat. The water-striders facing toward the pool and also those having the longitudinal axis of the body parallel with its margin found the water very promptly and with considerable directness, again suggesting the probability that they reached the pool according to the same principle involved in the case of *Vanessa antiopa* in finding the areas of bright sunlight. It must be recalled that the pool of water was, comparatively, of large size and that reflections of light from its surface would be more readily seen than from such a narrow brook as the one near White Heath. It is true that, in the experiments in which the gerrids faced away from the water, there was a little less promptness in reaching the surface of the pool and also some random movements. However, in this series of experiments, also, I believe that the sense of sight was the chief factor involved in assisting the gerrids to reach the water.

In the experiments when forty gerrids were used in each trial, I believe that vision played the chief rôle in directing them to the water, at distances both of one yard and of three yards. It is difficult to see what other factor could have served as a stimulus in assisting the water-striders to reach the pool with such directness and promptness as was displayed.

2. *Rôle of Moisture.*—During all of the experiments performed at Syracuse, it is possible that moisture, evaporating from the pool and diffusing through the atmosphere, served as an additional stimulus in effecting a positive response from the gerrids. Such a possibility is more feasible in this connection than was true in the case at White Heath, for the area of the water surface where the experiments were carried on at Syracuse is very much larger than the area of the water surface at the place in the brook where the experiments were conducted at White Heath.

## VIII. Summary and Conclusions

This paper treats of certain habitat responses of the large water-strider, *Gerris remigis* Say. The work was done partly near Urbana, Illinois, and partly near Syracuse, New York. Observations were made of the responses of the water-striders trapped in stream pools, during a period of severe drought, for the purpose of discovering what became of them after the pools dried up. These gerrids, being mainly apterous forms, were unable to migrate by flight. Experiments, related to the habitat responses, were performed for the purpose of finding out whether water-striders were able to reach their habitat, a brook of moderate size after having been removed from it and placed on the ground certain distances away.

In the late summer, during a severe drought, with a temperature from 90° to 100° F., water-striders, *Gerris remigis,* frequently were found on stream pools, connected by small riffles, at White Heath, near Urbana. As food became scarce or when a scum formed on the surface of some of the pools, the gerrids migrated, by way of the riffles, to other pools that were free from scum. As the drought progressed, the water-striders were congregated on the few pools that remained. Often the scum, a bacterial growth, killed large numbers of the gerrids.

These stream pools were studied and the responses of the water-striders were observed after the pools dried up.

For an entire day particular attention was directed to one small pool, dimensions $12 \times 5 \times \frac{1}{2}$ inches, which became dry at that time. There were twenty gerrids on its surface and they made no attempts to escape, as the pool rapidly became reduced in size.

After the pool had become entirely dry, the water-striders did not move away for a period of ten minutes. The initial locomotor responses were due, primarily, to the drying up of the water. This was the only change in external conditions and there was no other evident stimulus. Similarly, when gerrids were removed from aquaria, where they had been kept in captivity, they became very active when placed upon a solid surface away from the water. This was true even if previously they had been inactive. These water-striders moved with an awkward stumbling gait on land, but they made fairly rapid progress. Their methods of locomotion were by walking and jumping. Not infrequently, when jumping, the gerrids seemed to lose control of the orientation of the body, and sometimes made a turn of 180 degrees.

The gerrids responded readily to contact stimuli, which usually was evinced by them in coming to rest against pieces of dry mud, driftwood, stones, and clumps of dead leaves. They occasionally crawled underneath objects of the character that have been mentioned. They did not remain there permanently, for even after carefully marking the exact place, I never have been able to find them the following day. Shade and a lower temperature, combined with contact, probably were the factors which influenced the water-striders to stay quietly in such places. They did not burrow into the mud, nor into the banks of the brook for the purpose of æstivating until the drought had passed. So far as I was able to observe, the gerrids did not æstivate.

Ten out of the twenty gerrids, or 50 per cent., reached the nearest pool—dimensions 3 yds. $\times$ 2 yds. $\times$ 5 in.—which was ten yards down the dry bed of the brook away from the site of the former pool where the twenty water-

striders were entrapped. The first one to reach the pool did so in 5 minutes and 30 seconds. Another gerrid required fifteen minutes to find the water. The last water-strider to arrive at the pool completed the journey in forty minutes. There was considerable variation as to the time necessary to reach the pool on the part of the others, the average being 14 minutes and 30 seconds.

The direction of locomotion, of the ten water-striders that did not find the pool was mainly up the dry bed of the brook. Four wandered so far upstream that there was little probability of their reaching water. With reference to the six remaining gerrids, two of them jumped into a large crack in the dry mud of the brook channel; two crawled under some driftwood; one worked its way into a clump of dead leaves; and one disappeared while I was observing some other water-striders. The following day I was unable to find any of these gerrids, although I sought for them thoroughly, and had marked carefully the various places where they were seen last on the previous evening.

There was considerable variation, by different individual water-striders, as to the amount of time consumed in traversing the distance between their former abode and the large pool of water downstream. None of the gerrids, that reached the pool, journeyed there along a straight path. Those that were among the first to complete the journey seemed to make the least number of errors in direction. All of them made deviations from the most direct route, and also evinced random movements. They found the pool of water through a blundering method of trial and error. The responses of the gerrids that moved downstream and found the water and also of those that moved upstream and were not so successful were, in the main, very similar, although the latter, traveling a longer distance, made many more erratic movements. In general there appeared to be a lack of definiteness in orientation with reference to the direction of the pool and a lack of promptness in journeying to it.

There appeared to be a tendency on the part of the water-striders to keep moving along the path already taken, unless some other stimulus diverted them. This frequently occurred, and contact proved to be the commonest form of stimulus that brought about such diversion. They wandered along a certain path until some stimulus acted upon them. Then they changed their path and tried another direction. There were times when it was difficult to observe what was the stimulus causing the change in direction. In fact on certain occasions there appeared to be no new external stimulus, no change in the external environment, and yet there occurred a change in direction. Therefore the change in direction probably was due to some disturbance of the physiological condition of the animal brought about by some internal stimulation.

Fifty per cent. of the total number of gerrids entrapped on the surface of the stream pool were successful in reaching water elsewhere. In this instance the water was ten yards away from the site of the pool on which the insects were trapped. So large a number, I am confident, is very unusual, for several other observations of a similar character show that a very much smaller percentage were able to find water after the pools on which they were confined had become dry. In some cases the water was at distances of less than ten yards, while in other cases it was eleven, twelve, and fourteen yards distant. I believe that large numbers of apterous individuals die during periods of long and severe droughts. I have some evidence of this from out-of-door observations. Further, I have found that water-striders, frequently, soon die in the laboratory, when the water in aquaria was permitted to evaporate to dryness. This was true even if the temperature was not higher than 85° F.

Experiments were carried on near Urbana for the purpose of observing with what promptness and directness water-striders, *Gerris remigis*, returned to their habitat

after having been removed from it, and also for the purpose of observing their responses while doing so. Twenty water-striders were used at each trial. They were removed quietly from the surface-film and then carefully placed on the ground at distances of one, two, three, and four yards away from the brook. In some experiments the gerrids faced the water, in others they were parallel with the current of the brook, and in still others they faced away from the stream.

In all the experiments in which the gerrids faced the brook, the majority of them regained the surface-film. When they were placed on the ground one yard away from the water, all those that reached the brook did so in less than one minute. In no experiment were there more than two gerrids that did not reach the water. When the water-striders were taken two and three yards away from the stream, they were back again on its surface within 2 minutes and 30 seconds. Those gerrids that were placed on the ground four yards away from the brook displayed more random movements in reaching the water than did those that were nearer to it and a slightly smaller percentage succeeded in finding the brook. Those that reached the water did so within four minutes. The experiments with the gerrids parallel with the brook showed that the majority of them reached the water at distances from one to four yards inclusive. Some individuals required a little longer time to make the journey than did those that faced the brook. Sometimes there was a little delay before they began to jump toward the water. Those that were taken four yards away from the brook evinced more hesitancy and more random movements than was the case with the gerrids placed on the ground at points nearer the water. Experiments with the water-striders facing away from the brook showed again that the majority of the gerrids reached the water from all distances from one to four yards inclusive. There were more random movements and less promptness on the part of the water-striders in these experiments than

in any of the previous ones. A slightly smaller percentage reached the water from a distance of four yards than was the case in any of the other experiments.

Experiments were conducted in order to discover whether vision, moisture, or both of these factors functioned as stimuli in influencing the water-striders to find the brook. A barrier was constructed to shut off the view of the stream, but to be so arranged as still to permit moisture to pass through it. However, the barrier proved to be defective in this respect. The water-striders were a little less prompt in reaching the water when the barrier was employed than was the case when it was not used. The information that was obtained regarding the responses of the gerrids proved to be inconclusive. However, I am strongly of the opinion that vision is the important factor in directing these hemipterons to find water.

Experiments, of a character similar to those that previously have been described, were undertaken near a small rapid brook in the vicinity of Syracuse. Near the headwaters was a large pool, its approximate dimensions being $55 \times 17 \times 2$ feet, formed by an artificial dam and on its surface were thousands of gerrids. It was here that the experiments were performed.

The gerrids used in the experiments were taken directly from the surface-film of the pool. Different individuals were employed in each experiment. In all the experiments in which the responses of individuals were recorded, the distance from the pool to which the gerrids were taken was one yard. Six experiments were grouped together for convenience.

In the first, second, and third groups of experiments, the water-striders were placed on the ground facing away from the large pool. In the first group of experiments the total time consumed by all the gerrids in reaching the water was 12 minutes and 14 seconds. The average time required to find the pool was 2 minutes and $2\frac{1}{3}$ seconds. In Experiment VII, the gerrid had not yet reached the water after ten minutes had elapsed. Omitting this ex-

periment, the total time necessary for all the water-striders to reach the pool was 2 minutes and 14 seconds, and the average time consumed in finding the water was $26\frac{4}{5}$ seconds. In the second group of experiments the total time used by the water-striders to reach the pool was 3 minutes and 6 seconds. The average time required to reach the water was thirty-one seconds. In the third group of experiments the total amount of time that elapsed before all the gerrids had reached the water was 17 minutes and 55 seconds. The average time necessary for individuals to find the pool was 2 minutes and 30 seconds. The gerrid used in Experiment XXVI did not reach the pool and it was observed for 15 minutes and 25 seconds. If this experiment is omitted the total amount of time for all the water-striders to reach the pool was found to be 2 minutes and 30 seconds, while the average time required for the gerrids to find the water was thirty seconds. The results of these experiments are typical of many others. A large majority of the gerrids were successful in reaching the water, only two individuals out of eighteen failing to do so. There were a number of random and trial movements, but in the main, the gerrids returned to the surface-film with considerable promptness.

In the fourth, fifth, and sixth groups of experiments, the initial position of the gerrids was facing the water. With respect to the fourth group of experiments, all the water-striders consumed a total amount of time of 2 minutes and 40 seconds in reaching the surface-film. The average time necessary to return to the water was $26\frac{2}{3}$ seconds. The gerrid used in Experiment XXXIV consumed ninety seconds of time before it succeeded in finding the pool. Omitting this experiment the total amount of time required by all the water-striders to return to the brook was 1 minute and 10 seconds, while the average time neecssary to reach the water was fourteen seconds. In the fifth group of experiments, the total amount of time required by all the gerrids to find the pool was 1 minute

and 52 seconds, and the average time consumed was $18\frac{2}{3}$ seconds. In considering the sixth group of experiments, it was found that 1 minute and 24 seconds elapsed before all the gerrids were back on the surface-film and that the average time necessary to reach the water was fourteen seconds. The results of these experiments are, in the main, very similar to many others not recorded here. Out of a total of eighteen gerrids not one failed to get back to the water. The water-strider used in Experiment XXXIV was the only one that took an unusual amount of time to reach the pool. On the average, the gerrids used in the second three groups of experiments required, approximately, only about one half the amount of time that was required by the water-striders in the first three groups of experiments in order to reach the pool of water. The gerrids in the second three groups of experiments made fewer mistakes and a less number of random movements in finding the water than was the case in the first three groups of experiments.

Just a brief statement will be made with reference to a series of experiments in which the bodies of the gerrids were placed parallel to the shore of the pool. In other respects the experiments were similar to the groups of experiments, one to six inclusive, the results of which already have been recorded. In general, the results were very much like those obtained in the second group of experiments, with the exception that a little more time was required by the gerrids in reaching the water. There was not quite so much promptness, on the part of the water-striders, in moving toward the pool. They evinced a few more trial directions before arriving at the water and occasionally a gerrid did not succeed in reaching the pool.

A series of experiments was carried out in which the gerrids were not oriented, specifically, with reference to the pool of water. The individual responses were not considered in these experiments, as the water-striders were used in large numbers, but records were made of the number of gerrids that reached the pool and records also

were made of the length of time that was necessary to find the water. Forty gerrids were employed in each of the experiments, which well might be considered as mass experiments. They were placed on the ground one yard away from the water in a number of the experiments, and in the case of the other experiments, the water-striders were placed on the ground three yards away from the pool.

The results of the different experiments one yard away from the water were very similar in many instances. Therefore the data will be given of only one experiment. A great majority of the gerrids were back on the surface of the pool within fifteen seconds from the time they were placed on the ground. In thirty-five seconds all but two individuals had reached the water, and in one minute of time all the gerrids were striding back and forth on the surface-film. The water-striders jumped toward the pool with considerable promptness. They made comparatively few errors in direction and few random movements. Sometimes there was a gerrid that did not reach the pool.

In the experiments three yards distant from the pool of water, a great majority of the gerrids were back on the surface-film within forty seconds after they were placed on the ground. In the majority of these experiments, all the water-striders had returned to the water within 2 minutes and 5 seconds. A very few gerrids were not successful in reaching the water. A fair degree of promptness and directness were evinced by the water-striders in jumping toward the pool. There were, perhaps, more errors made in direction of movement than was the case with the gerrids in the experiments one yard away from the water.

In all these experiments conducted at the brook near Syracuse, it seemed probable that the sense of sight was the most important factor in directing the gerrids to the water, although moisture also may have exerted an influence on their responses.

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# SHORTER ARTICLES AND DISCUSSION

## SEX-CORRELATED COLORATION IN CHITON TUBERCULATUS[1]

1. Among mollusks the occurrence of clear-cut differential characters associated with sex is rare. Differences of size in the sexes of diœcious species are known, though in some instances the larger size of the female is a consequence of protandric hermaphroditism; there are also certain records of slight, and possibly inconstant, sex-differences in shell form; the hectocotylus of dibranchiate cephalopods, however, is almost the only well-defined instance of a "secondary sexual character" in mollusks —and this is an accessory organ of copulation. Color differences of this nature seem not to have been observed. Some importance may therefore be attached to the description of a pronounced color difference, correlated with sex, which has been found in the commonest placophoran at Bermuda, *Chiton tuberculatus* Linné, particularly since this differential coloration seems capable of interesting interpretation in several directions of theoretic importance.

2. In adult chitons of this species there is noticeable what appears at first sight to be a considerable diversity in the degree to which pigment, of a salmon-pink hue, is developed upon the foot and other soft parts exposed in ventral view. Somewhat less than half of the individuals have the foot, ctenidia, and other soft parts of a pale buff color; in the remainder, the foot, head, ctenidia and mantle are to various degrees tinged with salmon-pink or orange-red pigment, the color being in some cases startlingly vivid. This difference is most pronounced during late spring, but persists to some extent throughout the year. The pigmentation is not correlated in any way with size; individuals of any length from 3.4 to 9.2 cm. may be either pale buff or salmon-pink on the ventral surface; nor does the intensity of reddish pigmentation, when present, depend upon size. In dorsal view it is quite impossible to distinguish the two groups of animals, unless the plates be artificially separated to an extreme degree, and not even then with any certainty.

The differential coloration proves to be correlated with sex,

[1] Contributions from the Bermuda Biological Station for Research, No. 109.

in the sense that the soft parts of male chitons are never colored pink; whereas those of maturing females invariably are, the intensity of the pigmentation depending to a large extent upon the state of maturity of the ovary, to a lesser extent, it seems probable, upon the quantity and the kind of the algal food available in differing environments.

I have been at some pains to verify this conclusion by numerous dissections and by microscopic examination of smears from the gonad of 129 individuals. As in most chitons, the nature of the single median gonad is readily distinguishable, when mature or nearly so, owing to the fact that ovary and testis are differently colored. In the case of the young *C. tuberculatus*, and of the immature gonad in animals of all sizes, testis and ovary are macroscopically undistinguishable, being pigmented in the same degree by a brick-red substance, which will be referred to in what follows. These observations were made for the most part during the week ending March 30, 1918, at which time motile sperms and well-developed (but not mature) eggs were present. The ripe testis differs in color from the ovary because the amount of red pigment in the stroma of the male gonad does not increase after a very early stage; so that the testis comes to appear as a milk-white organ with innumerable interlacing threads of dull crimson upon its surface. In the ovary, on the contrary, the amount of this red substance increases enormously. No trace of a gonad was detected in 72 animals less than 3.4 cm. total length,[2] the smallest female being 3.4 cm., the smallest male 3.4 cm. also. A group of 67 individuals between 3.4 and 9.2 cm. length, collected at random, was examined by first carefully noting the coloration of the tissues (foot, etc.), then investigating the condition of the gonad. Among these 67, 27 were males containing active sperm; the foot, ctenidia, and other parts were in every case pale buff in color. The remaining 40 were clearly separable from the others by the presence of pink or orange pigment, and were without exception females. A further group of 64 chitons was first divided into two lots, "pale" and "pink," respectively; smears and teased preparations of the gonads showed that in only two instances was the expectation based upon the group first studied defeated, and in these instances the animals were small females with very immature ovaries. Summarizing the

[2] An investigation of the adult life-history of *C. tuberculatus*, to be described in detail elsewhere, shows that chitons of this size are at least two years old.

results from both groups, it was found that the 131 individuals with recognizable ovary or testis comprised 76 females and 55 males,[3] the females, when mature, being distinguishable externally by the development of a salmon-pink or orange-red coloration of the soft parts (foot, head, ctenidia, mantle).[4]

3. The color difference between the sexes of *Chiton* is believed to be of special significance, for the following reasons: because the coloration of the soft parts of the female is directly traceable to metabolic activities associated with the growth of the ovary; and because it provides an example of secondary sexual coloration which has no conceivable utility, but is, on the contrary, so far as color is concerned, of a thoroughly accidental nature.

Concerning the first point: the data previously summarized show the definite manner in which reddish pigmentation is correlated with sex; there are also the facts, (1) that in animals less than 3.4 cm. length there is no trace of any pink body pigmentation, (2) that the intensity of such pigmentation agrees with the state of development of the ovary, (3) that the blood of female chitons is mahogany-red or deep orange in color (that of males being dull yellow), and (4) that the reddish hue of blood, external tissues, and ovary is demonstrably due to the same pigment substance.

This pigment shows by its chemical behavior that it belongs to the group of carotin-like "lipochromes," and is unrelated to the hæmoglobin which colors the buccal musculature of both sexes. It has also an absorption spectrum—one band in the blue-green, another in the violet—of a kind supposed to be characteristic of the "lipochromes." The pigment is not soluble in water, but is dissolved by either 95 per cent. alcohol, acetone, xylol, or chloroform, and by the last named is extracted from alcohol after treatment with alkalies; it is quickly decolorized by standing in contact with air, in the light, and is bleached (after passing through a deep blue condition) to lemon yellow by strong nitric acid. Concentrated solutions are orange-red,

[3] These figures give a sex ratio of males: females: : 1: 1.38; this is probably too high a proportion of females for the whole population, but there seems undoubtedly to be, in some places, a preponderance of females. The matter is worthy of further attention, in relation to the breeding habits of *Chiton*.

[4] For certain purposes in which eggs are required to be uncontaminated with sperm, the external sex-difference in *Chiton* is a valuable aid in experimental work; not only are the eggs abundant, of fair size, and easily obtained, but males may be entirely excluded from the laboratory.

dilute solutions yellow. The lipochrome is present in the cœlomic fluids of the male *Chiton,* though in very small amount, and, as previously stated, is present in the stroma of the testis. In the female *Chiton* large quantities are present, in "solution," in the blood and cœlomic fluid, and in the ovary it is clearly associated with the great quantity of fat globules there present; not all of the fat globules are stained with the red pigment, multitudes of the smaller ones being uncolored by it.

It seems clear that we have here another case where the developing ovary is associated with "fat metabolism"; the red lipochrome, accompanying a large volume of fatty materials, is prominently concerned (perhaps by reason of its easy oxidation) in the growth of the ovary, and both pigment and fat are vastly important for the formation of sperms. The occurrence of the ovarian pigment in much higher concentration in the blood and juices of maturing females is comparable to the condition found by Steche[5] in certain moths, whereof the blood of the female was chemically differentiated in an obvious way from that of the male.

That the pigment is concerned in the metabolism of the ovary is shown by the fact that as the ovary becomes mature, but before it is fully so (*i. e.,* early in May), it becomes of a deep *green* color with imbedded streaks of maroon-red. In surface view the ovary is then green, like that of most chitons, but the salmon or deep orange coloration of the foot, muscle, blood, etc., does not change. Hence, if these animals were to be examined in summer, with the ovary nearly or quite mature, the causal connection between ovarian pigmentation and body pigmentation would hardly suggest itself immediately. It is easily shown, by extracting the pigment in acetone, that the green hue must be due to a relatively simple modification of the original red substance. Such extracts are orange-yellow, are decolorized by $HNO_3$, and give a green flocculent precipitate with alkalies. The ovarian eggs themselves, at first colorless, are found by the middle of May to have assumed a faint pink tinge, whereas toward the end of June they become deep green.

4. Regarding the accidental character of the reddish color in the tissues of adult female chitons, it is sufficient to point out that the foot, where this character is most conspicuous, remains throughout life firmly adherent to the substratum; the gills also

[5] I quote at second hand, from Doncaster, 1914, "The Determination of Sex," p. 101.

are brightly pigmented, but the girdle of the chiton is never raised more than a few millimeters from the surface upon which the animal may be resting, and while above water, in the intertidal zone, even this minute elevation occurs only along a short length of the mantle at a time. If it be answered that these chitons frequently creep over one another, it should be remembered that in the Placophora there are no tentacular eyes upon the head, and in the genus *Chiton* no extra pigmental megæsthete eyes upon the valves; so there is, after all, no opportunity for sex-recognition through color (a fantastic idea, for other reasons also). That the coloration of the soft parts is ever visible to other animals seems equally improbable. Certain small isopods (*Spharoma*) commonly frequent the mantle "chamber" of *Chiton tuberculatus,* but they are found indifferently in the company of either sex. It is necessary to conclude that, so far as *color* is concerned, the pink or orange hue of the body of the female *Chiton tuberculatus* is of no ethological significance; the nature of the pigment, its association with the growing ovary, its progressive changes in the ovary itself, and its presence in the blood, make of this case a most excellent illustration of the "metabolic-accident" conception of certain types of animal coloration.

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## ON THE ALKALINITY OF THE SEA WATER IN LAGOONS AT BERMUDA[1]

The present land-form of Bermuda, resembling in certain respects the configuration of many "coral" islands, was employed by Heilprin[2] as an example of atoll formation through basic subsidence. The southeastern segment of the proto-Bermuda land mass, now the only area above water, in addition exhibits three distinct "sounds," or lagoons: Great Sound, Harrington Sound, Castle Harbor. These lagoons Heilprin also conceived to have originated through local subsidences. Fewkes[3] had earlier considered the origin of these lagoons, stating his belief that they, as well as the form of the islands as a whole,

[1] Contributions from the Bermuda Station for Research, No. 114.

[2] Heilprin, A., 1889, "The Bermuda Islands," Philadelphia, [vi] + 231 pp., 17 pl.

[3] Fewkes, J. W., 1888, *Proc. Bost. Soc. Nat. Hist.,* Vol. 23, pp. 518–522.

were due to the erosive inroads of the sea. The collapse of caves and the general scouring action of tidal and other currents, as subsequently emphasized by Agassiz,[4] rather than very local basal subsidences, were thus regarded as the forces responsible for the lagoons.

So far as the general question of "coral" islands is concerned, it is sufficient to note that, strictly speaking, Bermuda is not, of course, a "coral" island at all, nor is its form that characteristic of the islands commonly so termed.[5] The problem, however, of this erosive action of the sea, its nature, and its rôle in the determination of land form in the case of a limestone island, is insistently presented by the enclosed lagoons to which I have referred. Murray's idea of the solvent action of natural waters in relation to the hollowing-out of lagoons and to the building of barrier reefs has lately been attacked from several aspects. Thus Mayer[6] has pointed out that at Tutuila (Samoa) and at Oahu, the surface waters draining into the sea are probably too alkaline, and contain too much calcium derived from the land, to be effective in dissolving the shoreward parts of the coral reef-flat. In the case of lagoons, a great number of influences are at work to control the erosion of the rock and the deposition and removal of silt.[7] The measurement of the alkalinity of the lagoon water provides but one of the factors requisite for analysis of the thoroughly heterogeneous equilibrium between the water and the limestone. Such determinations are nevertheless valuable, and during a recent residence at the Bermuda Biological Station I had the opportunity of carrying out estimations of this kind over a period of many months.[8] Attention was chiefly given to the alkalinity of the semi-enclosed waters as compared with that of the open ocean. The determinations were made colorimetrically, by means of thymolsulphonephthalein with borate standards,[9] and phenolphthalein. The alkaline reserve was not estimated.

[4] Agassiz, A., 1895, *Bull. M. C. Z., Harvard Coll.*, Vol. 26, pp. 205–281, 30 pl.

[5] Howe, M. A., 1912, *Science*, N. S., Vol. 35, pp. 837–842. Pirsson, L. V., 1914, *Amer. Jour. Sci.*, Ser. IV, Vol. 38, pp. 189–206.

[6] Mayer, A. G., 1917, *Proc. Nat. Acad. Sci.*, Vol. 3, pp. 522–526.

[7] Crozier, W. J., 1918, *Jour. Exp. Zoöl.*, Vol. 26, pp. 379–389. Mayer, A. G., 1918, Year Book, Carnegie Instn. Wash., for 1917, pp. 186.

[8] Apparatus for this work was obtained by a grant from the C. M. Warren Fund of the American Academy of Arts and Sciences.

[9] McClendon, J. F., Gault, C. C., and Mulholland, S., 1917, Publ. No. 251 Carnegie Instn. Wash., pp. 21–69.

Some distinct indications were had of a seasonal variation in $p_H$ of the enclosed waters, but tidal and other diurnal complications in the lagoons would make it necessary to institute a long series of studies for the complete description of this phenomenon.[10] The general fact was quite apparent that the "inside" water (*i. e.*, water within the sounds) was less alkaline than the "outside" water over the reef flats, the latter likewise less alkaline than the open ocean. Eight sets of estimations gave the $p_H$ of water taken at flood tide just beyond the outermost reefs to the west and northwestward of Bermuda, as 8.25 (21°–23°), at a salinity of 36.4 ± per mille, agreeing with that found by other observers for Atlantic water in this general region. The $p^H$ of the "outside" water was at different times observed to lie between 8.09 and 8.23. Within the sounds, however, the range noted was from 7.95 + to 8.15. The case of Harrington Sound, an almost completely enclosed body of water, is the most interesting. The waters of this lagoon are in communication with the outside sea through but one surface channel, a narrow cut at Flatt's Inlet; there is also a small amount of subterranean communication. Several specific examples will make clear the differences found. The figures refer to samples taken with a tube of pyrex glass from a depth of 2–3 feet below the surface. Samples obtained from depths of several fathoms ran in about the same way.

**Sept. 13th, 1917.**

Great Sound, 9:40 A.M. Tide ebbing, Water temp. 26.9°; air 27.5° $p_H$ 8.20
North shore, 9:55 A.M. Tide ebbing, Water temp. 26.8°; air 27.8° $p_H$ 8.22
Harrington Sd., 10:55 A.M. Tide ebbing, Water temp. 27.4°; air 27.8° $p_H$ 8.06

**Nov. 22d, 1917.**

Great Sound, 9:05 A.M. Tide low, Water temp. 19.8°; air 24.0° $p_H$ 8.08
North shore, 9:40 A.M. Tide low, $p_H$ 8.20
Harrington Sd., 10:45 A.M. Tide low, $p_H$ 7.95

Such results obviously speak for the view that the solution of limestone by the sea within such lagoons as Harrington Sound must be reckoned with. Exactly how important a part it plays in the final adjustment of the land form can not, of course, be said. The *color* of the sea water, I might note, varies in correlation with the $p^H$. Within the sounds, color-readings on the Forel scale[11] averaged 5.5 (17 per cent. yellow), whereas

[10] *Cf.* Moore, B., Prideaux, E. B. R., and Herdman, G. A., 1915, *Trans. Liverp. Biol. Soc.*, Vol. 29, p. 233. McClendon, J. F., 1918, *Publ.* No. 252, Carnegie Instn. Wash., pp. 213–264.

[11] Steuer, A., 1910, Planktonkunde, xv + 723 pp., 1 Taf. und 365 Abb., Leipzig. [Pp. 84–98.]

out over the reefs to the northward the color index was 3.9 (8.6 per cent. yellow), that of the ocean beyond the reefs about 3.5 (7 per cent. yellow).

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## A SIMPLE METHOD OF MEASURING THE RATE OF RESPIRATION OF SMALL ORGANISMS

In view of the widespread interest at the present time in the subject of respiration in the lower organisms, it is thought that the following simple method of measuring the rate of carbon dioxide production in small non-aquatic animals and plants may be found useful not only by teachers who desire a quantitative method suitable for class instruction but also by investigators who wish, without any material sacrifice in accuracy, considerably to simplify the various procedures at present followed in making determinations of small amount of $CO_2$. The apparatus required may readily be constructed by anyone in a few minutes out of materials easily obtainable, and with it, it is possible to measure, with a probable error well within the normal uncontrollable range of variation of the material likely to be studied, a few thousandths of a milligram of carbon dioxide—an amount equal to that given out at ordinary temperatures by a sprouting grain of wheat in perhaps three or four minutes and by a house-fly in one or two minutes. It would be relatively easy still further to increase the delicacy of the method, though the gain in sensitiveness would be at the expense of the simplicity which in its present form is its chief recommendation.

The method is based upon the well known indicator methods of Haas[1] and of Osterhout,[2] but unlike the first, it is applicable to small non-aquatic organisms, and unlike the second, it involves the use of apparatus so simple in construction that it can be duplicated any desired number of times and can therefore be used even by large classes of elementary students. Furthermore, provision is made not only for the comparison of relative rates of carbon dioxide production but also for the measurement of absolute amounts. Simplicity is secured by taking advantage of

[1] Haas, A. R., *Science*, 1916, XLIV, 105.

[2] Osterhout, W. J. V., *J. Gen. Physiology*, 1918, I, 17.

two well-known facts: (1) that the carbon dioxide content of out-of-door air varies only slightly from day to day, and scarcely at all during the course of an ordinary series of experiments and (2) that the distribution of a quantity of carbon dioxide between given amounts of water and air may readily be calculated from the known absorption coefficients for this gas at various temperatures, which may be obtained from the *Landolt-Börnstein Tabellen* or elsewhere. The first fact obviates the necessity of removing all of the carbon dioxide from the apparatus at the beginning of the experiment and the second makes it possible, with a minimum of trouble, to prepare standards for comparison which contain any desired amount of carbon dioxide, thus enabling measurements of quantity as well as of rate of production to be made.

The apparatus in its simplest form consists of a Nonsol test tube about 75 mm. by 10 mm., which has been drawn out in the Bunsen flame somewhat above its middle into a constriction approximately 30 mm. long and 4 mm. in diameter. It is closed with a well-rolled cork which has been thoroughly soaked in, and coated with, acid free paraffin. The indicator solution is placed in the lower portion of the tube; the constriction prevents it, when the tube is agitated, from splashing on the organism contained in the upper portion. To enable quantitative measurements to be made, a series of standard tubes is required in which known amounts of carbon dioxide have been added to the same indicator solution as that used in the apparatus just described. For the preparation of these standards the following device is employed. A Pyrex or Nonsol flask with a capacity of about 150 c.c. is fitted with a well rolled cork through which a hole is bored and one of the unaltered Nonsol test tubes forced in such a way that when the cork is in the flask the bottom end of the tube projects freely upwards and its lip fits against the small end of the cork. After being thus prepared, the sides and the lower surface of the cork are thoroughly coated with paraffin of the best quality, partly to prevent leaks but chiefly to protect the indicator solution from actual contact with the cork, which would be very likely to cause changes in its color. It is desirable, though not absolutely necessary, to have as many of the flasks with the prepared stoppers as the number of standards to be employed—usually three to five. The only additional pieces of apparatus required are a carbon dioxide generator, a box for comparing the colors of the indicator tubes such as is commonly used in colori-

metric methods of determining hydrogen ion concentration (a convenient form is supplied by the Hynson, Westcott and Dunning Co. of Baltimore, but it is easy to improvise one out of materials in hand in any laboratory) a fine-pointed pipette, a medium-sized test tube, a large flask, and a few pieces of glass tubing. For the pipette, tubing, etc., Pyrex or Nonsol glass should preferably be used; if ordinary glass be employed it should be coated with paraffin where it comes in contact with the solutions.

The first step in making a determination is the preparation of an indicator solution which is in exact equilibrium with the out-of-door air. This may conveniently be done as follows: A two-liter flask is filled with tap water, taken to an open window or out of doors and all of the water except about 100 c.c. is slowly poured out, great care being taken that neither the breath of the operator nor any currents of air from the laboratory come near it at this time. Enough of a concentrated solution of the indicator (phenolsulphonephthalein) is added to give a color of the proper intensity, the flask is stoppered and vigorously shaken for several minutes. If the tap water is not nearly in equilibrium with the carbon dioxide of the air, as shown by any decided change in color on shaking, the solution should be poured into a second flask from which water previously brought more nearly into equilibrium with the air has been emptied, and shaken for several minutes more. The solution, when in equilibrium with air at 16° C. (where the absorbtion coefficient is approximately equal to unity), and at a pressure of 760 mm. of mercury, contains approximately 0.3 c.c. of $CO_2$ per liter, or 0.0006 mg. per c.c. The exact amount need not be determined, however, since it is a constant quantity in all of the tubes used, and it is the amount *added to* it which is significant. To secure the benefit of the most sensitive part of the range of the indicator, the solution thus prepared should have a p H of approximately 7.6 to 7.8, *i.e.*, it should have a decided pink color with very little trace of orange. If the tap water is not alkaline enough to produce this result, a few drops of very weak NaOH may be added before the final shaking. If the tap water is too alkaline, it may be diluted with distilled water. The use of tap water rather than a weak solution of NaOH in distilled water is recommended merely for the sake of convenience and economy in those cases where it is suitable.

The next step is the preparation of the comparison tubes in which the indicator solutions, instead of being allowed to remain

in equilibrium with ordinary air and therefore to contain at 16° C. 0.0006 mg. of $CO_2$ per c.c., has received additions in the various tubes of known amounts of this gas. This is accomplished as follows: A current of pure $CO_2$ from the generator is allowed to pass through a few c.c. of distilled water in the medium sized test tube until the water is saturated at atmospheric pressure. It is convenient to use in the test tube a stopper with two openings, one for the inlet tube which carries the gas below the surface of the water and the other for an open glass tube projecting from the upper part of the tube through the cork and several inches into the air. This permits the excess gas to escape, and since $CO_2$ is heavier than air, the test tube soon becomes filled with a pure atmosphere of it, the outside air not readily entering through the long and narrow escape tube. It is necessary that the current of $CO_2$ shall be slow enough to give no more than atmospheric pressure in the test tube. If only a few c.c. of water at a time are charged, the added pressure of 2 or 3 cm. of water, due to the dipping of the inlet tube below its surface, is not significant. The minimum time required to saturate the water has not been determined, but it is the custom of the writer when fresh water is taken to allow the current to flow for at least thirty minutes; afterwards, by keeping the tube corked between experiments, the water remains almost saturated and exact equilibrium may easily be established in five or ten minutes. It is, of course, very important that neither the test tube nor the tube admitting the $CO_2$ shall give off appreciable amounts of alkali, hence the recommendation that Pyrex or Nonsol glass be used or the same result be secured with inferior glass by means of a thin coating of paraffin.

Having a solution whose $CO_2$ content can accurately be calculated if the temperature and the barometric pressure are known, the next step is to add measured amounts of it to successive portions of the indicator solution. This is done as follows. The first flask and the test tube in its stopper are filled with distilled water, which by shaking has been brought into equilibrium with out-of-door air, and emptied with the precautions already noted. Five c.c. of the prepared indicator solution are then added, the cork quickly inserted and the whole vigorously shaken. The color of the solution should not change if the proper precautions have been taken. The remaining flasks are then treated in the same way. By inverting them, the color of the indicator solution in their respective test tubes can be compared against a

white background. It should be the same in all. The first flask is kept as a control, nothing being added. It is inverted, and the cork with its test tube containing the indicator solution removed and quickly stoppered with a paraffined cork. To the second flask one drop of the carbon dioxide saturated water is added, to the second two drops, to the third four, or any desired number, etc. The stopper in each case is quickly replaced and the whole apparatus shaken vigorously until the $CO_2$ has distributed itself between the solution and the air. The test tubes are then removed and corked as described above. The amount of $CO_2$ added to each c.c. of the solution may now readily be calculated by taking into account the absorbtion coefficient for the temperature in question and the relative amounts of water and air. The volume of a single drop of the added solution has, of course, previously been determined by counting the number of drops required to give a volume of, for example, 5 c.c. In adding the $CO_2$ it is convenient to use a fine-pointed pipette which can be inserted in the escape tube of the test tube in which the water has been charged. This makes it unnecessary to remove the stopper of the latter or otherwise to disturb it. Unless the pipette is first filled with $CO_2$ (which may, however, readily be done and which is to be recommended), a little of the gas will escape from the free surface of the liquid within the pipette. If only a few drops are used from the lower portion of the pipette, however, no error will result if one works quickly enough. To drop the solution into the flasks without allowing any appreciable amounts of $CO_2$ to escape into the air requires a little practice, but after a few trials the best method is discovered and the necessary skill acquired.

When the standards have been prepared, which requires only a few minutes at the most, everything is ready for an actual measurement. The procedure is as follows. After filling the prepared test tube with out-of-door air, 1 c.c. of the indicator solution is placed in its lower portion and the organism to be studied in its upper portion, either free or attached to the cork by a loop of thread. The tube is closed and agitated gently, either continuously or at intervals, to mix the air and the indicator solution thoroughly. It is neither desirable nor necessary to shake the tube vigorously; with a little practise it will be discovered that a very slight movement of the proper sort will keep the liquid filled with bubbles and the air in the whole apparatus in circulation. From time to time the color of the solution is

compared with that of the standards. It is obvious that since the indicator solution is the same in each case and the starting point is the same, the same color indicates the same amount of $CO_2$ added. It is only necessary, from the relative volumes of water and air in the apparatus and the absorbtion coefficient under the conditions of the experiment, to calculate the total amount of $CO_2$ produced by the organism to the time of the observation. Since the absorption of the $CO_2$ by the solution lags a little behind its production, it is well not to consider the time from the starting point to the first tube; from the first to the second, however, and the second to the third, etc., this factor is approximately the same in each case and therefore does not appreciably affect the results.

As to the delicacy of the method, the indicator solution and the air in the tube, if the temperature is 16° C. and the absorption coefficient therefore equal to 1, each contain 0.0006 mg. of $CO_2$ per c.c. A ten per cent. increase causes a slight visible change in the color of the indicator solution, so a production of 0.0003 mg. in the 5 c.c. tube ought theoretically to be detectable. In practise, however, it is desirable to work with somewhat larger amounts, *e.g.*, 0.001 mg. or more. In measuring the time, it is well instead of trying to determine the point at which the two tubes exactly match to take the average between the last observation where the unknown tube is pinker and the first where it is yellower than the comparison tube. To meet a possible objection, it may be said that when the carbon dioxide has increased one hundred per cent., the oxygen in the tube has decreased only approximately 0.2 per cent.; consequently changes in the amounts of oxygen available for the organism during an ordinary experiment are hardly significant.

In conclusion, it may be stated that the method has been tested by comparing it with that of Lund[3] on the same organism (a firefly) and the differences obtained were only of the order of magnitude observed where two successive observations were made on the same individual by the latter method alone; that is, within the limits of uncontrollable normal variations of the species in question.

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[3] Lund, *Biol. Bull.*, 1919, XXXVI, 105.

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# ARE THE FACTORS OF HEREDITY ARRANGED IN A LINE?

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IN the February (1919) number of the *Proceedings of the National Academy of Sciences*, Professor Castle states that he has "shown that the arrangement of the genes in the sex-chromosome of *Drosophila ampelophila* is probably not linear, and a method has been developed for constructing a model of the experimentally determined linkage relationships."[1] This declaration is so widely at variance with the conclusions jointly agreed upon by all *Drosophila* workers, that the arguments or assumptions which it involves would seem to call for careful examination. It may be stated at the outset that the principle upon which Professor Castle constructs his models appears exceedingly direct and simple—it is merely to make a figure such that the distances between all the points represented on it are exactly proportional to the frequencies of separation actually found between the respective factors in the most reliable experiments. If this is done, Castle contends, the models will be three-dimensional instead of linear in shape.

1. The first argument which Castle gives against the view that the groups of genes (which he admits, at least

[1] Sturtevant, Bridges and Morgan also have published a defense of the view of linear linkage, in the *Proceedings* of the National Academy of Sciences (5, 1919, pp. 168–173) and Professor Castle has just replied to them in the same journal (5, 1919, pp. 501–506). It is believed that the present paper, although written and accepted for publication in the NATURALIST, previously to this article, meets all the points therein brought forward.

for purposes of argument, to be in the chromosomes) are linear, is that "it is doubtful . . . whether an elaborate organic molecule ever has a simple string-like form." This argument is therefore based upon the unique assumption that the whole chromosome (or that part of it containing the genes) consists of one huge molecule. Later, he speaks still more explicitly of this "chromosome molecule" and says, "the duplex linkage systems of a germ cell at the reduction division must be . . . twin organic molecules," so that "a purely mechanical theory [of crossing over] seems inadequate to account for interchange of equivalent parts between them." The argument may therefore be paraphrased as follows: since (1) the whole group of genes is but a single organic molecule, and since (2) an organic molecule can not be linear, then it must follow that (3) the group of genes is not linear, and that the theory of crossing over is therefore erroneous. Although the premises of this argument are both entirely gratuitous, it must be admitted that there is no flaw in the reasoning, once the premises are admitted.

2. The second argument brought forward against the linear arrangement of genes is that, in the linear maps, the distances between widely separated loci are not strictly proportional to the per cents of crossing over actually found, being relatively too large, in comparison with the per cents of crossing over. This he terms a "discrepancy" in the map, which has required the "subsidiary hypothesis" of double crossing over, in order to harmonize it with the theory of linear linkage. The answer to this is that it has never been claimed, in the theory of linear linkage, that the per cents of crossing over are actually proportional to the map distances: what has been stated is that the per cents of crossing over are *calculable* from the map distances—or, to put the matter in more mathematical terms, that the per cents of crossing over are a *function* of the distances of points from each other along a straight line. As will be shown presently, this circumstance alone is sufficient to

show that the factors must be bound together in a linear series; the precise nature of the function (involving coincidence, etc.) will then determine for us precisely the mode of incidence of the crossing over—*i. e.*, granted the linear series, it is then possible to calculate from the data the exact frequency of single crossing over, double crossing over of the various possible types, and multiple crossing over. Double crossing over thus becomes, not a "subsidiary hypothesis," but a phenomenon directly demonstrated.

It may, however, be noted in passing that, even if there had been no experimental evidence at all in regard to the nature of the linkage it could not have been conceded that Castle's alternative postulate—that no double crossing over can ever occur at all—would have been any more plausible *a priori* than that of the *Drosophila* workers which admits the existence of double crossing over. For, once the occurrence of single breaks in a chromosome is admitted—a point agreed upon by both sides—it is just as arbitrary to deny the possibility of double breaks as to assert their existence. Although Castle nowhere does explicitly admit that he has adopted this alternative "subsidiary hypothesis"—the denial of the possibility of double crossing over—yet an inspection of the theory of linkage which he himself has proposed shows that in this it has been tacitly assumed throughout, being necessary for the purposes of the solid models. Were double crossing over once admitted to occur, it could no longer be claimed that the distances between the factors in the three-dimensional models are exactly proportional to their per cents of separation—a condition which it is the sole aim of the existence of the models to fulfill.

We may now return to examine more carefully the main argument upon which linear arrangement and its corollaries (double crossing over, coincidence, etc.) is based. The fact previously stated that the linkage relations between the genes are such that they are all calculable from the positions of points in a linear series

may also be expressed as follows: given any three linked factors, A, B and C, if any two of the linkages between them are known—say, the linkage AB and BC—then the third linkage—AC—is determined (the most convenient practical method for calculating it is to make use of the "curve of coincidence" of the particular chromosome). This is, two of the linkage values may be taken as "independent variables" and the third is then "dependent" on them—in this sense we may say that B is linked directly to A and to C, but that A is only linked to C through the linkage of each of these factors with B. Since this is true of any combination of three-linked factors (ABC, BCD, CDE, ACD, etc.) it can be shown that the factors are all linked together in chain arrangement, any one factor being linked directly to only two others (those which we may regard as being on either side of it), its linkage with the rest being entirely dependent on these intermediary linkages.[1] This remains true as a discovered mathematical fact of the linkage relationships, shown first in experiments of Sturtevant's designed to investigate the problem, and this is what the writer has designated as "the law of linear linkage." Whether or not we regard the factors as lying in an actual material thread, it must on the basis of these findings be admitted that the forces holding them linked together—be they physical, "dynamic" or transcendental—are of such a nature that each factor is directly

[1] *I. e.*, all the linkages (factorial $(n-1)$ in number) between the $n$ factors in a group, can be shown to be dependent on (functions of) only $n-1$ "primary" or "independent" linkages. To obtain the most perfect expression of this dependency it is necessary to chose as the $n-1$ independent values the two strongest linkages involving each of the $n$ factors (what we should call linkages AB, BC, CD, DE, etc., as contrasted with AC, AD, AE, BD, etc.). On this system, the other linkages all become definitely determined, the secondary linkages being in each case a function involving the sum of certain of the primary linkages. If, however, the primary linkages are not chosen according to the above rule, so as to constitute a "chain formation," no formula can definitely express the relationships of the linkages, for the secondary linkages will then in some cases depend upon the sum, in other cases upon the difference between the linkages taken as primary.

bound, in segregation, with only two others—in bipolar fashion—so that the whole group, dynamically considered, is a chain. This does not necessarily mean that the spatial relations of the factors accord with these dynamic relations, for it is conceivable *a priori* that factor A might be far off from B, in another part of the cell, or that both might be diffused throughout the cell, and that they might nevertheless attract each other, during the segregation division, by some sort of chemical or physical influence. In the discussion that follows, no implication as to the actual physical arrangement of the genes is intended when the terms "linear series," "distance," etc., are used; these will refer only to the relations existing between the points in the linear map, which may be regarded merely as a mathematical mode of representation of the data themselves. It will be shown, however, at the conclusion of this article, that when the various conditions which have to be fulfilled at segregation are taken into consideration, any other explanation for these peculiarly linear linkage findings than an arrangement of the genes in a spatial, physical line proves to be hazardously fanciful.

In the case of the larger distances, in order to discover what function of the distance the per cent. of separation represents, it would be necessary to conduct extremely delicate determinations, involving very extensive data in experiments dealing with many points simultaneously. Nevertheless enough has been done to show that even for the larger distances the per cents of separation do depend on the distance in the linear map—being less than the distance by an amount which varies in a fairly regular manner according to the distance itself; hence it is known that the higher per cents of separation certainly involve some function of the linear distance.

In the case of the smaller distances, on the other hand, the function has been rather accurately ascertained; it is very close to the simplest one possible, that is, there is an almost exact proportionality here between the map

distances and the per cents of separations. Just as distance AB plus distance BC on a line are equal to distance AC, so the corresponding small frequency of separation between A and B, plus the small frequency between B and C, are found to be almost exactly equal to the frequency of separation between A and C; for this reason if the factors A, B and C are represented as points in a straight linear map, the distances between any two of them will represent the corresponding separation frequencies in an almost proportionate manner. A few examples of this principle are shown in Table I; it has been confirmed in innumerable other crosses, with many different factors. Moreover, it is found that the smaller the distance involved, the more exact is the porportionality that obtains, the less being the relative discrepancy between the frequency AC as found by experiment, and the value AC obtained, as on the map, by the summation of values AB and BC. The relationship which exists between the small separation values is hence just the sort which Castle himself would demand, for a proof of linear linkage. But whereas Castle would require this relationship to hold for all values, small or large, it may be shown that its existence in the case of the small alone is all that would be necessary for a complete proof of the doctrine of linear linkage, even if the large values were no sort of function of the linear series. For, if we proceed according to Castle's own method, and construct a map to represent the relations of the small values just described, showing each of the frequencies by a proportionate distance on the map, we necessarily obtain a map each section of which is practically a straight line. In the case, for example, of the data for v, g, and f, shown in Table I, if we represent the separation frequencies by proportionate distances in space, we must place point v at 10.7 units from g, and g at 11.3 units from f; if these two conditions are both to hold, then the only possible way of bringing f to its distance of 21.8 units from v is to put the three points in a nearly straight line, as shown

TABLE I

BACK CROSS OF FEMALES HETEROZYGOUS FOR v, g AND f. (PERFORMED BY BRIDGES; REPORTED BY WEINSTEIN)

| I. Non-separations | II. Separations of v from g and f | III. Separations of f from v and g | IV. Separations of g from v and f | V. Total Flies |
|---|---|---|---|---|
| 2651 | 360 | 380 | 3 | 3394 |

| Resultant Per Cent. of Separations Between v and g | Resultant Per Cent. of Separations Between g and f | Resultant Per Cent. of Separations Between v and f |
|---|---|---|
| $\frac{(II + IV)}{V}$ | $\frac{(III + IV)}{V}$ | $\frac{(II + III)}{V}$ |
| 10.7 | 11.3 | 21.8 |

in Fig. 1. Other results indicate that the line would be exactly straight if still smaller distances were studied. Enough data have been obtained in the case of chromosome I of *Drosophila* to determine in this way the "shape" of each part of the linkage group, and each part, by itself, is thus found to follow the rules for linear

FIG. 1. Direct representation of the linkages in Table I. (*vg*, *gf*, and *vf* are each represented by a line of length proportionate to the respective frequency of separation.) The dotted curve shows the "average angular deviation" of the factors from a straight line.

distances in an extraordinarily rigorous manner. That is, given the factors ABCDE, etc.,—or to take an actual case, y, w, A, bi, cl,—it is found that the linkages of y, w, and A are proportional to their distances in a straight line, so are the linkages of w, A, and bi, for A, bi, and cl, etc. But, since every part of the group is thus linear, it must then be true that the entire group is linear. A line all of the parts of which are straight is a straight line. Any differences then observed between the size of the larger distances and the per cents of crossing over, even if they were so irregular that they could not be thought of as a function of the linear system itself, would then have to be regarded as due to peculiarities in the incidence of the crossing over, superimposed upon a system of genes which was really linear in formation,—

modifications due to specific correlations between crossings over in different regions. But since, as has been stated, the differences between the larger per cents of crossing over and the linear distances are not unregulated, but do give clear evidence of being themselves a function of the map distances, these larger per cents of separation as well as the smaller ones can be used in proof of the linear system of linkage. The systematic differences between the frequencies and the map are hence due to double and other multiple cross overs, which vary in frequency in accordance with the distance involved.

It is true that a certain amount of the differences actually found between the larger frequencies and their corre-

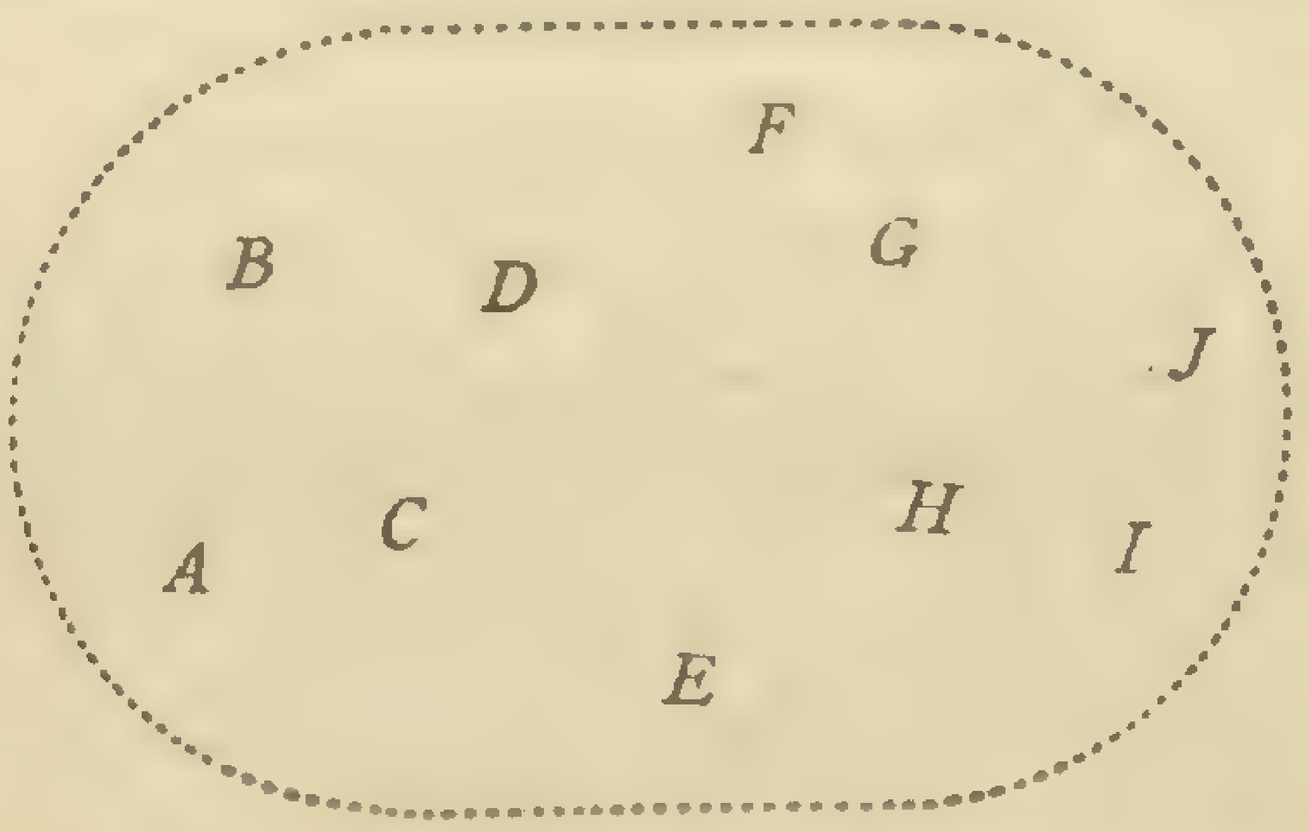


FIG. 2. Example of the sort of relations which would hold between factors arranged non-linearly (although in a plane figure). It is evident from this figure that more remote factors, such as *AE* and *J*, are likely to be arranged more nearly in a straight line than factors nearer together, such as *AB* and *C*.

sponding distances on a straight linear map might be thought of as due to the cumulation of minor discrepancies which existed between the small frequencies and distances but each one of which was by itself too small to be detected in the data for the smaller distances, being within the limits of experimental error. As the small discrepancies in such a case would, to be always cumulative, all have to have a bias in the same direction, this would amount to saying that the line along which the points were really disposed had a slow, even curve, too slight to be detected except when large distances were considered. The straight line would then be sufficiently accurate as a proportionate representation of all the

small values but not of the large ones. If the validity of the evenly curved figure were accepted, it would in no way disagree with the finding of a linear arrangement of the genes, but would merely substitute a curved line for a straight one. In a really non-linear figure, such as shown in Fig. 2, the relations between the smaller distances would be (if anything) less of a linear type than the relations between the larger distances,—factors further apart in a thick rod, for example, would have to be more in line than those near together. The fact that the opposite relation holds in the actual data shows conclusively that the factors are in some sort of a line. There is an *a priori* objection, however, to accepting a curved line as an explanation of the linkage relations, in that it is very difficult to imagine a plausible set of conditions in the chromosome which would hold the factors rigidly in this curved line but which would at the same time determine the number of separations between the factors according to their direct (straight) distances from each other, instead of according to their distances along this line. But, quite aside from *a priori* reasons, there is an experimental result absolutely fatal to the curved line "explanation"; this consists in the finding of those classes which are termed by the *Drosophila* workers "triple crossovers." In the case of these classes the separations are of such a type as to require the assumption of a break in the curved line at three points simultaneously. As it is obvious that a break in only one plane could not cut the curve at more than two points, the triple crossovers therefore would have to be due to a break in more than one plane. The occurrence of breaks in more than one plane, however, disturbs the assumed relation of simple proportionality between separation frequencies and map distances, which was the basic postulate upon which the curved line was constructed. If the distances are after all once admitted to be not exactly proportional to the separation frequencies, then there remains no reason to assume, just because the larger separation fre-

quencies are out of exact proportion to the distances on a straight linear map, that this is because the line is curved, and the factors thus nearer together. If still more evidence against the curved line idea be desired, it may be added that when the curve is constructed so as to be in good agreement (statistically) with the relations found between the smaller frequencies, it is then not sufficiently arched to permit the representation of the larger frequencies by proportionate distances (see section 4). A detailed compilation which I have made of all the data has shown that experimental error will not well account for the differences thus obtained between the two sets of results. The curved line being abandoned, it becomes, therefore, necessary to revert to "double crossing over," in explanation of the deviation of the large frequencies from the straight map values.[2]

3. If we examine further into Castle's argument, however, we find that he objects, not only because the larger separation frequencies are not proportional to the distances in linear maps, but also because he believes that the smaller frequencies are not proportional; in fact, according to his solid models, none of the kinds of frequencies, small or large, could even be a function of the distances in a linear map. In his solid, or rather, three-dimensional, models, which purport to have the factors so spaced that all distances between them are exactly proportional to the corresponding linkages, the factors are scattered about at all angles to each other, in such a

[2] The fact that the *geometrical* line which represents the linkages of the factors should be taken as straight does not imply that the supposed *physical* line in which the factors lie is straight. So long as the factors lie in any kind of physical line at all, then, if their linkages are determined, in some way, by their distances as measured *along* this line, these linkages should be representable on the basis of a straight geometrical map, inasmuch as all distances taken *along* a curved line must have the same interrelationships as distances in a straight line. Hence the curving of the chromosome filament is a matter entirely aside from the issue here involved, since the separation frequencies of the factors in the supposed filament are not conceived of as dependent upon their direct distances from each other but rather upon their distances along this filament. Thus the filament may, for these purposes, be treated as if it were straight.

way that their distances could never be represented as a function of distances in a single line. The cause of this discrepancy between Castle's figures and the relationships observed by the *Drosophila* workers lies in the nature of the data which Castle uses, or rather, in his manner of using the data. For Castle constructs his maps, or models, on all the data obtainable, indiscriminately, and regardless of the fact that most of the data for the linkage values involved have been secured in as many different experiments. On the contrary, it is necessary, in order to determine exactly the relationships existing between interdependent linkage values, that all the data be obtained from the same experiment. This is because the precise value obtained for any given linkage is not only subject to the ordinary error of random sampling but may vary significantly in different experiments, in response to different environmental conditions, the age of parents, genetic factors, and the amount of discrepancy due to differential viability. Piling up enormous counts does not eliminate these sources of variation. Any slight aberration thus produced in the absolute value of one of the linkages (say AC) will then alter so materially its relative value, as compared with the other linkages (AB and BC), obtained in two different experiments, that the different values no longer fit into the linear system; they will not be expressible as any sort of function of the system. That is why Castle found that the *Drosophila* workers' own data gave the per cent. of crossing over between y(yellow) and w(white) as 1.1, between w and bi (bifid) as 5.3, and between y and bi as 5.5, a relationship quite at odds with their claims concerning linear linkage for short distances. Castle could have pointed out numerous similar "discrepancies," by similarly choosing to compare exactly (within, say, one unit of distance), the results of different experiments. In fact, had we been allowed to select the experiments for him, we could have chosen values such as the following: Sb (frequency between star and black) 39.3;

bp (frequency between black and purple) 5.9; Sp 0.4. If Castle will follow his usual procedure here, and represent these frequencies by proportionate distances in a model, he will disprove not only linear linkage but both Euclidean and non-Euclidean geometry and plain arithmetic. The trouble in the case just cited arises in the fact that the first two values are those obtained under ordinary circumstances whereas the third is a value obtained in the presence of the factor CIIL which decreases enormously the amount of crossing over. Clearly it will be unfair to expect a single map to represent all three values simultaneously. Nevertheless, similar although less exaggerated, disturbing influences may be, and frequently are, at work causing discrepancies between the results of "ordinary" experiments, so that it should be evident that the latter are not ordinarily fit to be subjected to the delicate comparison which is necessary for the purpose of determining the nature of the linkage system.

To some critics, it might at first sight appear inconsistent for the *Drosophila* workers to use the above argument against Castle's system, in view of the fact that these workers themselves also combine the results of different experiments in constructing their chromosome maps. The answer to this is that the variations in linkage between ordinary experiments are usually so small absolutely, that, if all the data for independent linkage values, like AB, BC, CD, etc.—are joined together and represented in one linear map, the latter will be accurate enough for the usual purpose of computing approximately the per cents of separation: the factors will appear in their correct order, and with approximately the correct distances between them. If, however, a study of the nature of the system of linkage is to be made, much more precise knowledge than this is required, for it is necessary to know exactly the relative strengths of interdependent linkages—like AB, BC and AC—as compared with one another. In such a case the small absolute deviations occurring in the different experiments become

large relative deviations of the linkages as compared with each other—this is particularly true the smaller the absolute per cents of separation are—and so a totally erroneous impression of the nature of the linkage system may be produced. The nature of the linkage system—whether it is linear and, if so, what function of a line is involved—can only be studied to the best advantage in experiments involving several factors at the same time, but if our judgments regarding it have already been arrived at, or verified, in this way, it is then quite legitimate to use this knowledge for other factors, and to join the results of different experiments involving them into one linear map.

TABLE II

Separation Frequencies Between Every Two of the Six Sex-linked Factors y, bi, cl, v, s, B, as shown in a Count of 712 Flies from a Cross in which Twelve Sex-linked Factors were Followed Simultaneously. (Muller)

| Directness of the Linkage, According to the Linear Map | Factors Considered | Number of Separations | Per Cent. of Separations | Sum of Numbers of Separations of Each from an Intermediate Factor | Sum of Per Cents. of Separations of Each from an Intermediate Factor |
|---|---|---|---|---|---|
| "Primary" (for the experiment) | y and bi | 39 | 5.5 | | |
| | bi and cl | 53 | 7.4 | | |
| | cl and v | 112 | 15.7 | | |
| | v and s | 57 | 8.0 | | |
| | s and B | 95 | 13.3 | | |
| Dependent on two "primaries" | y and cl | 92 | 12.9 | y bi + bi cl = 92 | 12.9 |
| | bi and v | 165 | 23.1 | bi cl + cl v = 165 | 23.1 |
| | cl and s | 167 | 23.4 | cl v + v s = 169 | 23.7 |
| | v and B | 152 | 21.3 | v s + s B = 152 | 21.3 |
| Dependent on three "primaries" | y and v | 198 | 27.8 | y cl + cl v = 204 | 28.6 |
| | bi and s | 216 | 30.3 | bi v + v s = 222 | 31.2 |
| | cl and B | 240 | 33.7 | cl v + v B = 264 | 37.0 |
| Dependent on four "primaries" | y and s | 247 | 34.7 | y cl + cl s = 259 | 36.4 |
| | bi and B | 275 | 38.6 | bi v + v B = 317 | 44.5 |
| Dependent on five "primaries" | y and B | 296 | 41.6 | y v + v B = 350 | 49.2 |

In the experiments previously cited, the nature of the linkage in various sections of the chromosome has been

studied by following the inheritance of three factors in that region simultaneously. By a series of extensive counts of this sort the nature of the linkage in each individual section of the first chromosome has been studied, and found to be linear. The data in Table II are derived from an experiment which involves less extensive numbers than these, but illustrates to better advantage the linear behavior of all parts of the chromosome at once. These data are taken from Muller's cross of flies heterozygous for twelve mutant sex-linked factors. The results for six of these factors—those scattered most evenly along the chromosome—are shown in the table, which gives the number and per cent. of separation between every one of these factors and each of the other five. It will be seen that it happened that in this particular experiment, for all per cents of separation below 23, the per cent. of separation between any two factors was exactly equal to the sum of the per cents of separation of each from a third factor lying between them, whereas for factors less closely linked, the larger per cent. was less than the sum of the other two by an amount varying closely with the size of the large frequency itself. The data obtained in this same experiment for the three factors y, w and bi are given separately in Table III, in order that they may be compared to better advantage with the non-linear relation for these factors which Castle claims, as a result of his combination into one map of the results of separate experiments. Whereas Castle obtained a triangular figure to represent the three frequencies (y w 1.1, w bi 5.3, and y bi 5.5) it is seen that in this experiment, where all three were followed at the same time, an exactly linear relationship was obtained (y w 1.7, w bi 3.8, y bi 5.5). An experiment of Sturtevant's involving just these three factors is shown in the same table (III); here too the relations are entirely linear. In like manner the values obtained in the 12-factor experiment for the loci of y w and A are given in Table IV (y w 1.7, w A 1.4, y A 3.1) to be compared by the

"triangular" values (y w 1.1, w A 1.7, y A 2.0) claimed by Castle. The numbers in these experiments are quite sufficient to have revealed clearly any such triangular relationships as shown in the data chosen and figured by Castle.

TABLE III

SEPARATION FREQUENCIES OF y, w AND bi

*A. Data from the same experiment as that which furnished Table II. (Muller)*

| I. Non-separations | II. Separations of y from w and bi | III. Separations of bi from y and w | IV. Separations of w from y and bi | V. Total Flies |
|---|---|---|---|---|
| 673 | 12 | 27 | 0 | 712 |

| Resultant Per Cent. of Separations Between y and w | Resultant Per Cent. of Separations Between w and bi | Resultant Per Cent. of Separations Between y and bi |
|---|---|---|
| $\frac{(II+IV)}{V}$ | $\frac{(III+IV)}{V}$ | $\frac{(II+III)}{V}$ |
| 1.7 | 3.8 | 5.5 |

*B. Data from a cross involving just these three factors. (Sturtevant)*

| I. Non-separations | II. Separations of y from w and bi | III. Separations of bi from y and w | IV. Separations of w from y and bi | V. Total Flies |
|---|---|---|---|---|
| 487 | 3 | 16 | 0 | 506 |

| Resultant Per Cent. of Separations Between y and w | Resultant Per Cent. of Separations Between w and bi | Resultant Per Cent. of Separations Between y and bi |
|---|---|---|
| $\frac{(II+IV)}{V}$ | $\frac{(III+IV)}{V}$ | $\frac{(II+III)}{V}$ |
| 0.6 | 3.2 | 3.8 |

TABLE IV

SEPARATION FREQUENCIES OF y, w AND A.

*(From the same experiment as that which furnished Tables II and IIIA. Muller)*

| I. Non-separations | II. Separations of y from w and A | III. Separations of A from y and w | IV. Separations of w from y and A | V. Total Flies |
|---|---|---|---|---|
| 690 | 12 | 10 | 0 | 712 |

| Resultant Per Cent. of Separations Between y and w | Resultant Per Cent. of Separations Between w and A | Resultant Per Cent. of Separations Between y and A |
|---|---|---|
| $\frac{(II+IV)}{V}$ | $\frac{(III+IV)}{V}$ | $\frac{(II+III)}{V}$ |
| 1.7 | 1.4 | 3.1 |

4. Although it has been shown that the linkage relations existing among the factors in any one experiment are functions of a linear series it might still be questioned whether there might not, after all, be some advantage in using Castle's system of graphic representa-

tion—whereby each separation frequency is supposed to be shown by an exactly proportionate distance on the figure, no matter how many dimensions may be required for this purpose. It will now be shown, however, that such a system of representation is impossible, quite aside from the fact that the models shown in Castle's papers are based upon data which can not legitimately be combined together. That is, no matter whether the data used are all derived from one experiment, or whether the results of different experiments are combined according to Castle's method, they could not be represented either in a three-dimensional or in any other geometrical figure, in such a way that all the distances would be proportional to the separation frequencies.

This may be illustrated by the data reported in Table II. It has been seen that the per cent. of separations between y and cl is exactly equal to the per cent. of separations between y and bi plus that between bi and cl. If then we represent these frequencies by actual distances, we must make the distance between points y and cl exactly equal to the distance between y and bi plus that between bi and cl. The only possible way to do this, on any kind of geometry—one-dimensional, three-dimensional or *n*-dimensional—it to put these three points in one straight line. In a similar manner we must place bi cl v in a straight line, and also v s B. Cl v and s are in almost a straight line, but there would have to be a slight bend at v, owing to the fact that cl s is very slightly shorter than cl v plus v s (on account of just one double crossover having occurred between them); this is correlated with the fact that cl s is a longer distance than the others considered. The figure so constructed, on the basis of Castle's own methods, is shown in Fig. 3; it is quite evident that this is the only figure which will represent directly (proportionately) the frequencies above considered. If, however, we now measure the distance on this figure between the extreme points, y and B, we find that it turns out to be 49.3, or very nearly the sum of

the intermediate distances (50.0), whereas the frequency of separation found between y and B in the actual experiment is 41.6. Similarly, the "model" shows too high a frequency for the other longer distances involved. (The long distances y s and bi B are 36.2 and 43.9 respectively on the model, but only 34.6 and 38.6 in the data; the moderately long distances y v, bi s, and cl B

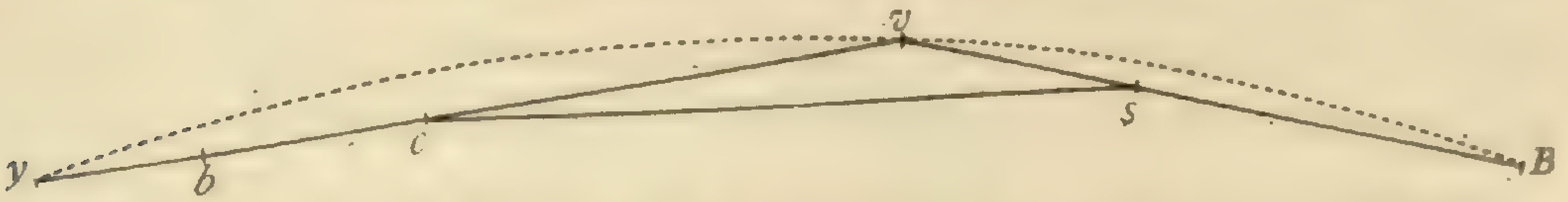


FIG. 3. Direct representation of the strongest and second strongest linkages in Table II. (*y bi, bi cl, cl v, v s, s B,* and *y cl, bi v, cb s, v B,* are each represented by a line of length proportionate to the respective frequency of separation.) The dotted curve shows the "average angular deviation" of the line of factors, according to this system.

are 28.6, 30.8, and 36.6, respectively, on the model, but 27.8, 30.3, and 33.7 in the data.) It would, on the other hand, have been possible to bring y and B close enough together in the diagram, and at the same time have adjacent factors the correct distance apart, by giving the line a curve, or bending it, as shown in Fig. 4. But if this is

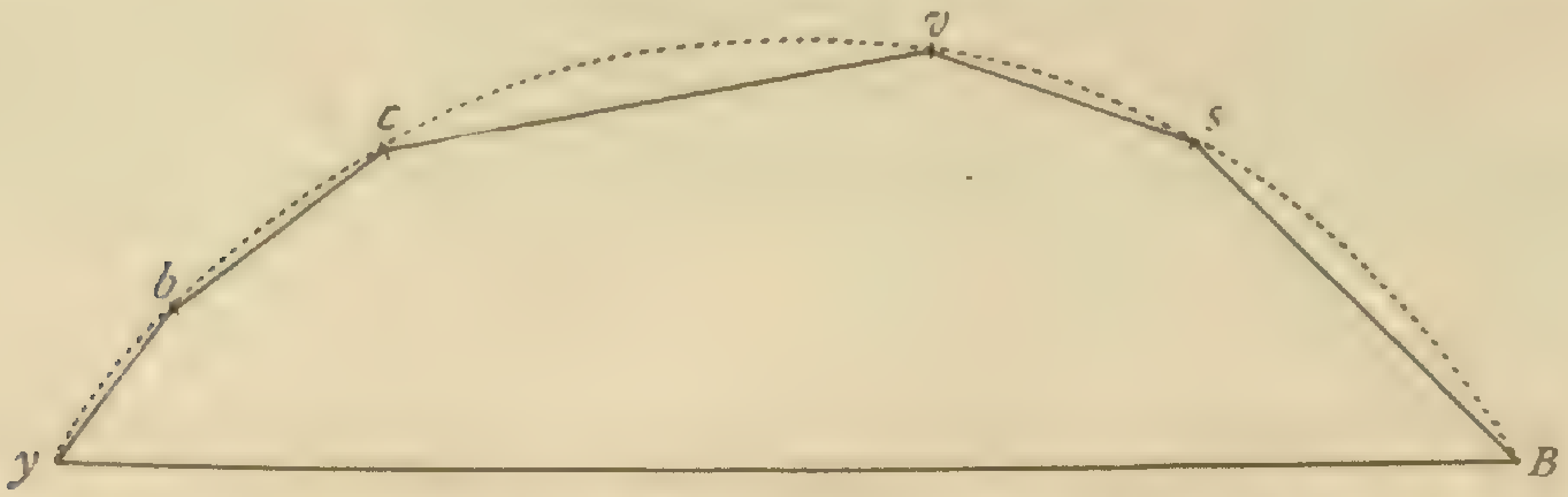


FIG. 4. Direct representation of the strongest and weakest linkages in Table II. (*y bi, bi cb, cb v, v s, s B,* and *y B* are each represented by a line of length proportionate to the respective frequency of separation.) The dotted curve shows the "average angular deviation" of the line of factors, according to this system.

done it is found that the distances of intermediate length (y cl, bi v, cl s, and v B) are not properly represented, all of them being relatively too short on the diagram. It would be unsafe to attribute these discrepancies, so uniform in direction, to the "errors of random sampling." The present experiment is cited, however, purely

as an illustration, to show what kind of discrepancies are meant. Discrepancies of exactly the same character and direction appear when the diagrams obtained by this method from experiments involving three points close together are compared with those from other experiments having three points far apart; that is, the former figures are repeatedly found to be nearer in form to a straight line than the latter; in such three-point experiments, moreover, highly extensive counts have been made, involving altogether (in the published experiments on the first chromosome), approximately a hundred thousand flies, and thousands of double cross overs.

5. The relation which has just been described, whereby the larger frequencies of separation are relatively smaller than could be directly represented in a curve constructed on the basis of the small frequencies, is due, according to the phraseology of the *Drosophila* workers, to the fact that the relative frequency of double crossing over ("coincidence") is so much larger for large frequencies than for small ones. Castle realizes to a certain extent the difficulty which this circumstance entails for his models, and he endeavors to meet it by means of the "subsidiary hypothesis" that the breaks in his models are more frequent in certain directions than in others. This assumption would, in some measure, explain away in a formal manner certain of the discrepancies (although cases of "triple crossing over" still remain an insurmountable obstacle), but the adopting of any such hypothesis really amounts to cutting away the ground from under the main theory of "proportionate representation," for the hypothesis involves an abandonment of the claim that the model represents each frequency by a proportionate distance between the nodes. For it is evident that if, in a given region, breaks in one direction are more frequent than in another, then points in this region which are an equal distance apart will be separated with different frequency according to the di-

rection of the line joining them. A given distance then no longer represents a given frequency.

6. It has been shown in the above two sections that a single figure will not represent accurately, by proportionate distances, the various linkage frequencies actually found in experiments involving many factors at once. If, on the other hand, it had been attempted to combine into one map the absolute frequencies obtained in a series of different experiments with two factors at a time, as Castle claims to do, the number and extent of discrepancies irreconcilable with any possible geometrical figure would have been much greater still. For, since the absolute frequencies found in different experiments necessarily have all sorts of irregular relationships to each other, it follows that it would be even less possible to show in one solid model separation frequencies which were obtained in this way. One such irreconcilable value has already been recognized by Castle—namely, the frequency y B. He is forced to represent this frequency by a curved wire in his model, because it is longer (being 47) than the longest distance possible (41) between these two points in any figure founded on a proportionate representation of the other frequencies. Of course, if distance on the model is to have any meaning, it cannot arbitrarily be represented along a straight line in some instances, and along lines having various degrees of curvature in other instances. In this one case, then, Castle is compelled to assume that the wrong value has been obtained, owing to experimental error, even though in all other cases he has assumed that it is quite legitimate to combine the results of different experiments. It would have been strange if, in making a model of this sort representing the separation frequencies of so many combinations of factors, Castle had not encountered more of these refractory cases. He does not mention any more, but it is noticeable that several other curved lines appear in his model. Moreover there is a conspicuous absence in the model of the factor lethal 2.

It would have been inconvenient to represent the observed linkage results for this factor by proportionate lines in the model, for, according to the results, lethal 2 would have to be placed at 9.6 from w and 17.7 from v, thus making the distance between w and v not greater than 27.3, whereas the established distance between w and v themselves is as much as 30.5; similarly, although only 9.6 from w, lethal 2 must be placed only 15.5 from m, although w and m are known to be at least 33.2 units from each other. Either the wv and wm lines would have to be considerably curved, therefore, or the lines between lethal 2 and the other factors would have to be *stretched* in some way—perhaps dotted lines would meet the difficulty!

Although any scheme of representing linkage results by exactly proportionate distances encounters the contradictions discussed above, it is noticeable that nearly all the most extreme departures from a plane curved figure (that figure which *comes nearest* to representing, by strictly *proportionate* distances, the ratios resulting from that type of linear linkage which actually exists) occur in the case of factors whose linkage ratios are distorted by differential viability or difficult classification. This is why the factors A, fr, sh, cl, bi, and the lethals stand out from the fairly regular curved line which Castle's models would otherwise conform to. The first two of the above factors are uncertain of classification, the others mentioned affect viability markedly. In the case of nearly all the remaining factors of the model, even though the results were taken from different experiments, it was nevertheless found, when the data were plotted, that they agreed pretty well with the expectations based on linear linkage. Moreover, the better the experimental conditions are in regard to viability, certainty of classification, and size of counts,—the more closely are these synthesized results of individual experiments found to coincide with the linear findings of experiments involving three or more points simulta-

neously. (It is for this reason that Sturtevant was first able to hit upon the general fact of linear linkage, on the basis of numerous careful experiments involving only two factors at a time.)

7. Owing to the inherent inconsistencies of the methods that were used to construct the solid models, it is to be expected that any predictions regarding separation frequencies which are deduced from them would be extremely unsafe. Castle states, however, that if any newly discovered gene has been located in the model, by obtaining its frequencies of separation from any three of the other genes contained therein, then the relation of the new gene "to all the others could be predicted by direct measurement from the model." In the case of two of the four predictions which Castle has made in this way, some evidence concerning the distance between the loci involved is already in existence.

One of the frequencies of separation in question is that between the loci of the recessive mutant factors glazed eye and rugose eye, in *Drosophila virilis*. Castle predicts, on the basis of his model, that the per cent. of crossing over between them should be found to be 4 or 5, or "probably a little greater." The work of Metz, and unpublished work of Weinstein, have shown, however, that hybrid females which carry both mutant factors exhibit the somatic character sterility possessed by the more extreme mutant type. When this dominance of an ordinarily recessive character in $F_1$ is taken together with the close similarity between the unusual effects produced by the two mutant factors (both produce a similar, peculiar effect on the eye, which is sex-limited, being more marked in the males), and with the fact that there is also a third mutant member of the series, with similar peculiar effects and similar linkage relations, it becomes highly probable that these factors are all allelomorphs. In that case they occupy "identical loci," and the frequency of separation between them must be 0. A direct determination of the per cent. of crossing over between

them is obviously impossible to obtain, on account of the sterility of the females carrying both factors.

Another prediction based on the solid models dealt with the frequency of separation between the factors hairy and magenta, of *Drosophila virilis*. It had been found by Metz that the per cent. of cross overs between hairy and forked was 3.1 and between forked and magenta 3.7; this would make the per cent. for hairy magenta 6.8 (or 0.6), if the factors were in a straight line. In the solid model, however, the arrangement of these factors, based on separate determinations of their frequencies of crossing over with distant loci,—is shown as triangular; and on the basis of this model Castle predicts that the frequency hairy-magenta will be found to be 4 or 5. The frequency has recently been determined by Weinstein, who has kindly consented to allow its use in this connection. He finds it to be 6.6.

It should be pointed out that Castle has endeavored to protect himself, in these predictions, by saying that they only hold, provided the relations given "have been determined with sufficient accuracy"!

8. One of Castle's specific objections to the linear maps, on which he lays much stress, is that on them the distances between the extreme factors is much more than 50 units, whereas factors which are linked must have a separation frequency of less than 50 per cent. It is only necessary to point out here that since, as we have seen, the linear maps, unlike the models, do not imply a proportionate relationship between the distances and the separation frequencies, these distances of over 50 do not connote separation frequencies of over 50 per cent. On account of the progressive reduction in separation frequency, due to double crossing over, that occurs with increasing distance, even distances of 100 or 110 in the second chromosome do not connote separation frequencies as high as 50 per cent. On the other hand, it should also be remarked that separation frequencies of over 50 per cent. would not be impossible *a priori*, as Castle

maintains; consequently any system of representing linkage which permitted or showed such values would not be *ipso facto* inconsistent. The mere fact that all factors hitherto worked with in a single chromosome have less than 50 per cent. of separation, and that those in different chromosomes have just 50 per cent., does not mean that factors can never be found which are so far apart, and which lie in such a rigid chromosome (little double crossing over) that they separate more often than they remain together at segregation. Whether this phenomenon should then be called linkage is but a question of words; the chromosomes themselves would have no regard for the 50.0 per cent. mark, or for the idiosyncrasies of our terminology.

The proof of the law of linear linkage, including all the main aspects of it which have been given above, has been stated on several previous occasions. It seems unfortunate that the argument has had to be repeated each time that a new "theory of crossing over" has arisen, for the discussion and data given in the original papers supply all the material necessary for a decision of the matter, at least so far as the germ plasm of *Drosophila* is concerned.

Before closing, it may be desirable to supplement these arguments for a mathematically linear mode of linkage, by a statement of the considerations which indicate that this mathematically linear linkage can have its basis only in a linear physical connection between the genes.

If the genes are not spatially arranged, or physically connected, in the same linear sequence as that in which they have been found to attract each other in linkage, then the forces of linkage attraction must be such as to "act at a distance." But, although acting at a distance, these linkage forces must nevertheless be extraordinarily specific—binding each gene directly to just two specific associate genes. Hence the forces could not be of an electrical nature, for, since there are only two kinds of electricity, electric forces could not be specific enough.

Similarly, the attractions could not be magnetic, nor could they be due to any kind of diffuse "physical" forces, such as those that emanate from centers of surface tension change or from centers of vibrational disturbances. Those who deny linear arrangement, while admitting the mathematically linear linkage results would therefore be driven to assume that the linkage attraction depended on the specific chemical nature of the genes, which, by virtue of their chemical composition, exerted a specific attraction at a distance, as the substances of adsorption compounds are sometimes supposed to do. But such a theory, as a method of accounting for linkage, becomes stretched to the breaking point when it is remembered that each gene must be assumed to have such an attraction for just two of the others, never more nor less, and that when this attraction is broken it is always exchanged for that of the allelomorph. Moreover, it would be exceedingly hard to reconcile this theory with the finding that changes in the nature of the genes—mutations—alter in nowise the sequence of their linkage attractions, and very rarely change even the strength of the linkages. And when we come to analyze the linkage relations in detail, and encounter the phenomenon of interference, we find relations that are entirely at variance with all our preconceptions concerning chemical attractions or chemical activity in general,—results that would force us to assume (1) that a breakage of the attraction between two genes leads to an increased attraction between the other genes and (2) that the amount of this increased attraction ("interference") depends solely on the directness of the connection ("distance") between these other genes and the one whose attraction was broken, being not at all influenced by the chemical nature of the broken attraction, or by the chemical nature of the other attractions themselves. The facts of "interference" or "coincidence" are thus diametrically opposed to a chemical view of linkage, although they, like all the other facts of linkage, are quite in accord with

ideas of a spatial, physical linear arrangement, their interpretation on the latter basis being natural and obvious.

The idea that the genes are bound together in line, in order of their linkage, by material, solid connections thus remains as the only interpretation which fits the genetic findings. In view of the additional fact that the chromosomes—themselves known to be specifically linked to the factor groups—can, at certain stages of their history, be seen to have the linear structure required, it would indeed be rash to adopt a different theory, without most cogent evidence of a startlingly new character.

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# INFLUENCE OF THE MALE IN THE PRODUCTION OF HUMAN TWINS[1]

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It is frequently pointed out that the father of twins can have little influence in determining their production; such production is purely a maternal quality, due to double ovulation. One possible way, however, in which the male may influence twin production is recognized, but this affects only 1-egg twins. Thus, if we assume that 1-egg twins are due to an early fission of the embryonic blastodisc, or if they are due to a secondary budding (following the method of the armadillo), then the sperm cell might carry the tendency to such fission or budding, as well as the egg cell. This possibility, however, does not help the statistical student of plural births, such as Weinberg, because he believes that the tendency to 1-egg twins is not inherited at all.

In the following study, there will be considered only the class of cases showing heredity most clearly, namely, those in which the principal fraternity under consideration has more than one pair of twins. Parents of such fraternities are spoken of in what follows as repeater fathers or mothers. Our query is then: "What is the relative importance in twinning of inheritance from the maternal and paternal sides, or what is the relative occurrence of twin labors in the close relatives of repeating mothers and of their husbands?"

To get an answer to this question, all available figures on twin repeaters were studied statistically. Of 355 labors occurring to the mothers of repeating mothers, 16 (4.5 per cent.) were twin labors. Of 289 labors occurring to the mothers of twin-repeating fathers, 12 (4.2 per cent.) were twin labors. These statistics thus indicate

[1] Read before the American Society of Naturalists, at Princeton, Dec. 30, 1919.

that the frequency of twins in the fraternities of fathers of twins is almost the same as that of twins in the fraternities of mothers of twins. Since the average proportion of labors which are twin labors is 1.1 per cent. for the population as a whole, we see that twins occur in the fraternities of repeating fathers as well as repeating mothers about four times as frequently as in the population as a whole.

To make use of more extended pedigrees, we may compare the tendency to have twin children on the part of *sisters* of the father and the mother of twin fraternities and on the part of *brothers* of the fathers and mothers of such fraternities. Then we obtain the results shown in the following table:

| | Per. Cent. of Births that are Twin Births |
|---|---|
| Father's sisters' children | 8.2 |
| Mother's sisters' children | 5.5 |
| Father's brothers' children | 6.5 |
| Mother's brothers' children | 4.5 |

From this table, most of the items of which were based upon ten or more twin labors, it appears that the sisters of twin-producing parents are more apt to have twins than the brothers of twin-producing parents; but the sisters of twin-producing fathers are more apt to have twins than the sisters of twin-producing mothers; also the brothers of twin-producing fathers are more apt to have twins than the brothers of twin-producing mothers. In all cases the proportion of twin births is very high, ranging from 4 to 7.5 times the average proportion of twin births in the whole population. These statistics then indicate that there is no important difference in the hereditary influence to twin production on the part of the father and the mother of offspring which include two or more sets of twins.

If, instead of considering the cases of twins in general, we pick out those of certain (or highly probable) *identical* twins, then we find, in 30 families with such twins, that the mothers came from fraternities in which (in 77 labors) there were 13 per cent. twin labors, and the fathers came from fraternities in which (in 38 labors) there were 13

per cent. twin labors. Here we see that there is an eqality of the maternal and paternal influence and that there is a larger proportion of relatives of *identical-twin* producers who are twins than of producers of *twins in general*. Indeed, the occurrence of twin-offspring to the fraternities of the parents of identical-twin producers is proportionally 12 times as common as in the population at large.

Another way of testing the inheritableness of 1-egg twins is by getting the frequency-distribution of the sex of twins in repeater families—those in which the influence of heredity most clearly shows itself. In these, therefore, we expect nearly an equality of twins of similar sex and of dissimilar sex, provided 1-egg twins are not found in these clearly inheritable strains. In 160 pairs of twins in repeater families, of which the sex is given, there are 54 of unlike sex and 106 of like sex. Expectation in the case of binovular twins is that there will be an equality of like and unlike sexed twins. Any excess of like-sexed twins is to be ascribed to the occurrence of 1-egg twins. In the present case, there is an excess of 52 pairs of like-sexed twins out of 160 pairs of twins, which indicates that about 1 in 3 of the twins in repeater families are identical twins, and this agrees approximately with statistics obtained from the population as a whole. From this we reach the conclusion that the tendency to production of 1-egg twins is certainly not less common in the case of repeater families than in the case of families in which there is only a single pair of twins. The statement, therefore, that there is no hereditary influence to be detected in the case of 1-egg twins appears certainly to be incorrect. In fact, the presence of heredity is more striking than in the case of other twins and this leads us to conclude that the hereditary tendencies toward uniovular multiple production so obvious in armadillo (*Tatusia*) persists also in man.

Still another way of testing the relative influence of the mother and father in twin production is the comparison of cases in which the father of twins has married twice, and the mother of twins has married twice. An examination of our records showed 30 families where at least one

parent of twins has married twice. In 14 cases it was the father who married twice, in 15 cases the mother, and, in 1 case, both father and mother. In the 14 cases of father of twins who had married twice, there were twins by both marriages in 2 cases, or 14 per cent. of all such cases. In the 15 cases where the mothers of twins had married twice, there were twins by both marriages in 3 cases, or 21 per cent. of all such marriages. The numbers are small, but, so far as they go, in view of the average occurrence of twins in only about 2 per cent. of all marriages (and hence if chance only were at work in 4 per 10,000 of both pairs of double marriages), they indicate that the tendency to twin production is hereditary and also that not only the mothers but also the fathers have great influence in determining the production of twins.

All the foregoing statistics speak strongly for the view that the father has about as much influence in the production of twins as the mother. This result at first sight seems quite inexplicable and indeed to reduce the whole matter to an absurdity. If twin production is due simply to double ovulation, what can the father have to do with the result?

The present paper does not attempt to give a final answer to this inquiry. It attempts only to set forth a hypothesis which suggests a line of experimentation to answer the question more definitely. We have assumed that 2-egg twins are due to the simultaneous bursting of two Graafian follicles while single births result from the bursting of a single follicle. There is, however, a good deal of evidence that single births are not always the consequence of the bursting of a single follicle merely. There are indeed several other factors that determine a single birth, such as the failure of one of two simultaneously expelled eggs to be fertilized or the failure of one of two simultaneously expelled fertilized eggs to develop to maturity. That is, it may well be that two eggs are simultaneously ovulated much more frequently than at present recognized and that the comparative rarity of twin-births is due either (1) to a failure of fertilization of one egg or (2) to a failure of development of one egg.

The conviction that not all eggs that are ovulated are fertilized is borne upon one who compares the number of corpora lutea in mammals that have large litters and the number of embryos that one finds in the uterus. I have recently made a number of counts in this respect in the case of sows and give below results in tabular form:

| Observation Number | Number of Recent Corpora Lutea | Number of Embryos Found | Average Length of Embryos |
|---|---|---|---|
| 1 | 3 | 3 | 15 cm. |
| 2 | 6 | 3 | 10 cm. |
| 7 | 8 | 7 | 6.5 cm. |
| 12 | 9 | 2 | 2.5 cm. |
| 13 | 8 | 7 | |
| | 34 | 22 | |

Thus from 34 corpora lutea, or 34 eggs expelled, only 22 embryos were found, counting only those which had reached a length of 2 cm., at which stage the chorion is already so large that it seems improbable that it should have been overlooked.

There is some reason for thinking that in humans also a certain proportion of the eggs ovulated fail of fertilization even in families in which there is no prudential restriction—in which the size of the families indicates a probability that nearly the maximum number of eggs became fertilized. Conclusions are fortified by the examination of a good genealogy including families of children born in the latter half of the eighteenth and the early part of the nineteenth centuries. Thus in a genealogy of the Gorton family, seventh generation, the intervals in round years between births in various fraternities (all related as cousins, are:

13 children—3, 1, 1, 5, 1, 2, 2, 2, 2, 2, 1, 5; all born 1795–1821.

10 children—2, 2, 2, 2, 2, 3, 2, 2, 2. In this case there is no unexpectedly large interval.

11 children—2, 2, 2, 2, 2, 2, 2, 2, 1, 5.

6 children—4, 2, 4, 3, 4; all born between 1792-1809.

8 children—2, 2, 2, 2, 2, 5, 2; all born between 1796–1813.

13 children—1, 2, 1, 2, 2, 2, 3, 3, 3, 2, 2, 4. In this case also

there seems to be no failure of fertilization, except at the end of the series.

9 children—4, 2, 2, 2, 2, 2, 2, 2.
10 children—3, 1, 4, 2, 1, 2, 2, 2, 2.
9 children—1, 2, 3, 4, 3, 2, 2, 3; born between 1825–1845.

One gets the impression that the normal interval between births, assuming all eggs to be fertilized, is about 2 years. The frequent intervals of 3, 4, 5 and even more years probably correspond to failure to fertilize, although they may be due to miscarriages or even in some cases to prolonged absence of the husband. In view of the fact, however, that we have to do here with a prevailingly rural population, chiefly farmers and millers in central New York State, the latter contingency is improbable.

The failure of fertilized eggs to complete their development is a real factor that must be taken into account. Attention has been called to the importance of this factor by John Hammond (*Journal of Agricultural Science*, VI, 1914) who has studied fetuses of rabbits and pigs and finds among them many degenerating individuals. Thus the number of degenerating fetuses in a large number of uterine horns examined varied from 0 to 19 per cent. I can confirm these results by observation made upon the uterus of a sow (No. 3) in which there were 2 corpora lutea in the left ovary and 5 in the right. In the left horn of the uterus there was a well-developed embryo 8 mm. long and one, evidently blighted, of 4 mm. The outlines of the latter embryo were highly abnormal and shrunken. The right horn of the uterus contained one embryo, 25 mm. long, a second 9 mm. long, and a third 6 mm. long. Thus with 7 corpora lutea in the ovaries, there were only 5 embryos found, of which one was completely blighted, another at 6 mm. length would probably soon have ceased development and two others at 8 and 9 mm. were far behind the best developed embryo, already 25 mm. long.

Work on yellow mice, of which the yellow × yellow matings give rise to 25 p. c. atretic embryos, and the far more extensive experience of Morgan with lethal factors in *Drosophila*, indicate that failure of development is a far

more common phenomenon than hitherto appreciated. Lethal factors, it may be pointed out, are a probable solution of one of the mysteries of gynecology; namely, that a woman who is sterile with one husband is often fertile with another, even when examination has shown no defect in the spermatozoa. Similarly a husband may have no children by one wife, but one or more by a second marriage. Parallel phenomena are common in dairy cattle. We conclude then that lethal factors are probably widespread phenomena even in human germ cells, and account for a certain proportion of long intervals between births, of early miscarriages, and of sterile unions.

The application of the foregoing two principles of failure of fertilization and failure of development to the question of the rôle of the male in twin production is now fairly obvious. More eggs are laid, even without prudential restraint, than come to development, and this is true not only of eggs laid successively but of eggs laid simultaneously; that is, twins that are born are the residuum of a greater number of twins that are started in their development and of a still greater number of pairs of eggs simultaneously ovulated.

The literature of gynecology is indeed full of cases of blighted twins. In a fairly large proportion of all twin births, one of the twins has remained at a stage of development of the third, fourth, or even earlier month. The fetus is often found compressed and flattened; the name is given of papyraceus twin. The number of blighted twins which have been referred to in the literature amounts to several score, but naturally is a very small proportion of the whole. The vast majority of blighted twins are simply lost unnoted with the afterbirth. A record is made only of the larger blighted fetuses; the others are entirely overlooked, since search is rarely made for undeveloped embryos in the afterbirth, and the birth is consequently regarded as a single one. We must believe that a certain proportion, perhaps a large proportion, of the fraternities which show two or three twin labors interspersed with single labors are those in which pairs of eggs

have been ovulated in each case, but one of the pair has failed to develop, either through failure of fertilization or early blighting.

Now the lethal factors show their influence first in certain combinations, just as in the matings of yellow × yellow mice. The $\frac{1}{4}$ of the embryos which die are those which are derived from germ cells containing the genes for yellow, whereas the other $\frac{3}{4}$ may develop fully. So we concude that among humans the cases of twin-repeating fraternities are those in which there are no or few lethal factors in the germ cells, so that there is a maximum fertilization and development of the eggs laid.[1] In the case of families comprising only one pair of twins, combined with a number of single births, it is probable that in other cases there had been a double ovulation but one of the pair had failed to develop. The additional fact to be taken into account is that twins are found in a higher ratio in large families than in small ones. Large families, however, connote high fertility of the male as well as the female. From all these facts we reach the conclusion that families which readily produce twins do so not only because in the mother the eggs were laid in pairs, but also because in the father the sperm is active, abundant and without lethal factors, so that the number of eggs fertilized and brought to full term approaches a maximum. To repeat, such fathers, experience indicates, belong to strains which are exceptionally fertile and in which twins are repeatedly produced both along male and female lines. Thus it comes about that the fathers of twins are about as apt to belong to twin-producing strains as mothers of twins and that twinning depends on constitutional—hereditary—factors on both sides of the house.

[1] F. H. A. Marshall (1910), "Physiology of Reproduction," p. 618, recognizes that certain abortions in sheep "may be due to a want of vitality on the part of the developing embryo." Similarly gynecologists recognize that a part of the 10 per cent. of barren marriages, and many of the early miscarriages, have no explanation in pathology, but apparently only in physiology.

# INHERITANCE OF CONGENITAL PALSY IN GUINEA-PIGS[1]

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## Introductory

In 1914 a litter of two guinea-pigs was born in our laboratory, one of which differed from the other in that it appeared to lack nervous control. When this individual was placed on its feet, attempts on its part to walk resulted in spasmodic stiffening of the legs, causing it to fall over on its side, where it lay helpless and unable to get up. Although the animal appeared otherwise in good physical condition, it was thought at the time that the trouble might be due to temporary nutritional disurbance, and attempts were accordingly made to feed it by hand,

[1] Papers from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin, No. 18. Published with the approval of the Director of the Station.

until it should be able to nurse. The effort was, however, unavailing, the subject gradually becoming weaker and the symptoms more pronounced, until death ensued in a few days. A second litter was produced by this pair on May 19, 1914. This consisted of three young, which appeared in all respects normal. On August 27, however, another litter of two was produced and one of these was like the abnormal individual described above. This gave rise to the suspicion that the defect might be due to some hereditary cause and consequently the mating of the same parents was continued, with the following results: November 6, 1914, one defective offspring; March 24, 1915, three offspring, 2 being normal and one born dead; and June 20, 1915, 2 normal, 1 defective and 1 born dead. Not counting the two born dead, since their condition with respect to normal reactions could not be determined, this pair then produced a total of 13 young, of which 9 were normal and 4 defective. These results not only strengthened the presumption that we had to deal with a heritable condition, but were so close to a three-to-one ratio as to suggest that it might be a simple Mendelian recessive. It may be stated at this point that further extensive experiments have proven conclusively the correctness of both of these presumptions.

Full discussion of the symptoms and of related conditions in man and other animals will be reserved until the experimental results have been presented. It is sufficient to say here that the defective condition is always clearly marked and easily recognizable, and that in no case have there been doubtful intermediates. Furthermore, such efforts as have been made as yet to rear the defective offspring have been uniformly unsuccessful; these individuals always die within a short time, usually within two weeks of birth.

## Origin of Palsied Stock

Later in 1915 palsied offspring were produced by other parents and studies of the pedigrees have shown that such individuals have appeared in three distinct lines

which are unrelated so far as the pedigrees show back to our original stock. This stock came from two sources, a few animals received from Professor Castle, of Harvard University, and somewhat less than a dozen young animals supplied us by our veterinary department, but obtained from a dealer. This stock had multiplied to about forty individuals at the time records were begun on it. The pedigrees show that in all probability there was only one individual, a male, in the Castle stock which might have brought in the palsy character, and since Professor Castle informs us that he has never noticed it in his animals, this individual may with considerable certainty be ruled out as the source of the defect in our experiments. The young animals received from the dealer were all of about the same age and were white spotted, very similar in appearance, which suggests that they may have been related. We are accordingly led to conclude that the character was introduced with this stock and that in all probability it may have traced back to one, or at least only a few, heterozygous animals, and that, furthermore, if there were more than one they were probably related.

## Inheritance of the Palsy Character

The factor for normality appears to be completely dominant and we have found it impossible to distinguish animals carrying the defective trait from those which do not on the basis of observable behavior or any other characters. The only method of separating the two classes is therefore by breeding tests. Owing to the fact that the affected (recessive) individuals always die, it has been necessary to conduct the experimental tests by the roundabout method of always mating animals to be tested to others known to be heterozygous. If the individual being tested was a homozygous normal no defective offspring would be produced by such a mating, whereas if it was heterozygous they should appear in the usual ratio for Mendelian recessives. We have therefore conducted extensive experiments to determine, (1) the ratio of palsied

offspring when two heterozygotes are mated, (2) the proportion of homozygous to heterozygous individuals among the normal offspring of such matings, and (3) the ratio of homozygous to heterozygous offspring when homozygote was mated to a heterozygote.

Since for practical reasons the number of offspring which can be produced from any particular pair of parents is limited, it became necessary to set a definite arbitrary number which should be taken as the minimum to indicate a fair probability that the animal being tested was homozygous for normality if no recessive young were born. Five was chosen as this minimum, but in every instance larger numbers were obtained where possible. All cases in which less than five normal offspring were obtained without the appearance of a recessive are discarded from the calculations. Furthermore, only six of the thirty individuals rated as homozygous normals on the basis of their breeding behavior had so few as five offspring, and in most cases the number was considerably larger, as is shown in Table I.

TABLE I

ANIMALS RATED AS HOMOZYGOUS NORMAL AND THE NUMBER OF EXCLUSIVELY NORMAL OFFSPRING ON WHICH THE RATING WAS BASED

| No. of Animals Tested | No. of Normal Offspring Produced by Each | Total Offspring |
|---|---|---|
| 6[2] | 5 | 30 |
| 3[2] | 6 | 18 |
| 1[2] | 7 | 7 |
| 1 | 8 | 8 |
| 1 | 9 | 9 |
| 4 | 10 | 40 |
| 6 | 11 | 66 |
| 1 | 12 | 12 |
| 1 | 13 | 13 |
| 2 | 14 | 28 |
| 1 | 15 | 15 |
| 1 | 23 | 23 |
| 1 | 24 | 24 |
| 1 | 26 | 26 |
| Total 30 | — | 319 |

[2] Eight of these ten animals died before more offspring could be obtained, one was discarded because of being a poor breeder, and one for some unassigned cause.

Further evidence that this test is fairly reliable is furnished by the fact that in matings of heterozygote to heterozygote affected offspring appeared in litters before five normal young had been born in 84 per cent. of the cases, while in 22 of the 32 matings the recessive appeared in the first litter. The complete data are given in Table II.

TABLE II

| Number of Matings | Number of Normal Offspring Produced before Litter Containing Recessive |
|---|---|
| 22 | 0 |
| 3 | 1 |
| 2 | 4 |
| 3 | 5 |
| 2 | 8 |
| 32 | |

For present purposes we have adopted the symbol $N$ to represent a factor for normality; the recessive, palsied animal is therefore $nn$.

1. *Ratio of Palsied Offspring when two Heterozygotes are mated.*—As there appears to be no need of presenting the detailed data of individual matings, the combined results of mating heterozygous animals together are given in the left hand side of Table III. Of the total number

TABLE III

Matings $Nn \times Nn$

| | Offspring | | | Tested Normal Offspring | |
|---|---|---|---|---|---|
| | $N$ | $nn$ | Born Dead | $NN$ | $Nn$ |
| Observed | 183 | 63 | 36 | 7 | 15 |
| Expected | 184.5 | 61.5 | — | 7.3 | 14.6 |

of offspring alive when born (that is, when found) 183 were normal and 63 palsied, an almost exact three-to-one ratio. We, therefore, feel safe in our assumption that the palsied condition is based on a single unit factor difference. The question might be raised as to whether the rather large number of offspring "born dead" might not represent a disproportionate number of palsied young. This does not, however, seem probable for a number of

reasons. In the first place, the palsied animals appear as strong and vigorous when born, and in all respects except for the nervous condition as fully developed as the normal young. This is further borne out by the weights, the weight of the palsied young being as great, indeed averaging slightly more at birth than that of the normals. The weights at birth of the living young from $Nn \times Nn$ matings are shown in Table IV, from which it will be seen

TABLE IV

WEIGHTS AT BIRTH OF LIVE OFFSPRING PRODUCED BY MATINGS $Nn \times Nn$

| | Number Weighed | Average Weight in Grams | Number Not Weighed | Total Individuals |
|---|---|---|---|---|
| Normal males | 91 | 88.61 | 4 | 95 |
| Normal females | 79 | 88.68 | 9 | 88 |
| Normal males and females combined | 170 | 88.65 | 13 | 183 |
| Palsied males | 32 | 92.21 | 0 | 32 |
| Palsied females | 29 | 87.96 | 2 | 31 |
| Palsied males and females combined | 61 | 90.19 | 2 | 63 |

that 170 normal offspring averaged 88.65 grams, whereas 61 palsied young average 90.19 grams. The slightly greater weight of the latter is probably not significant. These facts seem to indicate strongly that the congenital death rate was not differential with respect to palsy.

2. *Proportion of Homozygous to Heterozygous Individuals among the Normal Offspring of $Nn \times Nn$ Matings.*—Further proof that we were dealing with a single factor difference was provided by tests of the normal offspring from matings of heterozygote to heterozygote. These should, of course, consist of two heterozygous individuals to each extracted homozygous dominant, which should breed as free from the defect as any animals from non-palsy stock. As shown in the right-hand half of Table III, 22 of the 183 normal individuals were tested, of which 7 proved to be $NN$ and 15 $Nn$, the theoretical expectations being 7.3 and 14.6, respectively.

3. *Ratio of Homozygous to Heterozygous Offspring from Mating $NN \times Nn$.*—One other type of test was

made, namely, of the normal offspring resulting from the mating of homozygous to heterozygous individuals. The expectation in this case is equality of the classes, and the

TABLE V

MATINGS $NN \times Nn$

| | Offspring | | | Tested Offspring | |
|---|---|---|---|---|---|
| | $N$ | $nn$ | Born Dead | $NN$ | $Nn$ |
| Observed | 319 | .. | 36 | 14 | 11 |
| Expected | 319 | 0 | .. | 12.5 | 12.5 |

actual numbers found in the 25 tests made were 14 $NN$ and 11 $Nn$, the expectation in this case being 12.5 in each class (see Table V).

Additional evidence that the extracted homozygous normals are free from palsy "taint" is furnished by three matings of such animals together, from which 31 living offspring have been obtained, all normal.

The foregoing data would appear to be sufficient in number and in closeness of ratios to demonstrate conclusively that congenital palsy in guinea-pigs is inherited in simple Mendelian fashion and depends on a single unit difference, the normal condition being completely dominant to the heterozygote.

## SYMPTOMS

A brief description of the typical symptoms has already been given, but for comparison with the same or similar conditions which may be observed by others, it seems desirable to describe the symptoms of the palsy as it occurs in our stock in somewhat greater detail.[3]

A word ought perhaps to be said at this point about the use of the term congenital palsy. The congenital part is evident enough and needs no explanation further than to point out that we use it in the sense of being present at

[3] We wish to express our appreciation of valuable advice and assistance rendered us by Dr. W. J. Meek in connection with this and the following sections of this paper.

time of birth rather than of being contracted at time of birth, which is the connotation sometimes implied in relation to certain infectious diseases. The word palsy is used in the general sense to indicate the broad similarity of the condition in the guinea-pigs to trembling palsy in man. The term is intended to be a neutral one with no implications as to the ultimate cause of the disturbance. The condition perhaps in some ways more closely resembles tetany as manifested in mammals below man, but this term has been avoided as having possibly too specific an implication.

There is considerable variation in the degree to which different individuals are affected. In most cases the victim when discovered shortly after birth is lying on its side slowly moving the legs, twisting the body and lifting the head as if in a vain endeavor to get on its feet. The movement of the fore part of the body, head and forelegs is much more pronounced than that of the hind quarters and hind legs. Some individuals if placed gently on their feet are able to stand, though usually in a strained tense attitude. The difference between this and the normal position may be observed in Fig. 1, ♀ 1089.1 being a palsied individual, while the others are its normal brother and sisters. The photograph is taken from directly above. The affected individual has the feet somewhat spread and the body slightly contorted, while the others are in natural easy attitudes.

If left quietly to itself after being placed on its feet the animal usually stands unsteadily for a few moments and then when it starts to walk falls on its side, with characteristic movements of the legs to be described presently. Some animals are so little affected at birth that they are able with effort to gain their feet themselves, and to walk about in a clumsy, jerky, paralytic fashion. They experience the most difficulty in the control of the hind legs, which appear to be in a hypertonic state and are commonly moved more in a hopping fashion than in steps. A rough classification of 51 palsied animals soon after birth gives the following: 14 unable to rise and unable to stand

when placed on their feet; 18 able to stand but unable to walk; 5 able to walk when placed on their feet but unable to arise unaided; and 14 able to get up and to walk. It should be recalled that in all cases the symptoms grow progressively worse, leading to the most severe conditions, and to death in a week or two at most.

Breathing appears to be normal, as is also control of the muscles of the jaws and throat, for the less affected animals sometimes eat solid food, and those that are able to walk may suckle the mothers. Such individuals increase in weight for a time as rapidly as normal young, but with the progress of the disease they become unable to obtain nourishment, and consequently decline. We are unable to state at present whether death is attributable finally to starvation, or whether it is a direct sequel of the disease.

The most striking phenomenon in connection with the disease is the reaction to stimuli, particularly to auditory stimuli. This may be best observed in animals that can stand when placed on their feet but are able to walk only with great difficulty, if at all. If such an animal is placed on its feet and a sharp sound is then made, such as clapping the hands, snapping the fingers, or squeaking with the lips, the reaction is definite and immediate—the subject jumps upward and forward, due to a sudden stiffening, particularly of the hind legs, then falls on its side, the whole body shaking to some extent, but the legs exhibiting strong clonic spasms. To the same stimulus normal individuals give merely a slight start, and then sit unconcernedly as before. This result is clearly shown in Fig. 2, which depicts the same litter as Fig. 1, but following a stimulus which has thrown the affected individual into a spasm as described. Fig. 3 is a short time-exposure of an animal in a spasm lying on its side. The photograph shows clearly the movement of the feet.

Visual stimuli have relatively little effect in producing the above-mentioned reaction. Even if the hand is brought rapidly down to near the animal's eyes it seldom responds. The same is true for mechanical stimuli, the reaction occurring only if the stimulation is severe. Af-

1 ♂1089.3 NN ♀1089.4 Nn ♀1089.1 nn ♀1089.2 Nn

2 1089.3 1089.4 1089.1 1089.2

3 758.1

INHERITANCE OF CONGENITAL PALSY IN GUINEA-PIGS.

fected animals which are fairly able to walk may not fall over even as a result of an auditory stimulus. They give a jump, much more pronounced than the start of normal individuals, but manage to stay on their feet. Furthermore, even the more affected ones become less sensitive to repeated stimulation, and may after several reactions fail to respond sufficiently to make them lose their balance.

In the more severe stages the reaction appears to simulate intentional tremor, in that it follows attempts at voluntary movements of the hind legs. In less severe cases the animal can use the legs if free from other nervous excitation. It would appear therefore that the condition is induced by sudden nervous excitement, the degree of the stimulus necessary to cause complete lack of muscular control depending on the stage of progress of the affection.

The severe spasms commonly last but a few moments. If a guinea-pig stiffened out in one of the spasms is taken in the hand it can soon be felt to relax, following which it either lies quiet or makes slow movements of the head and to some extent of the legs as previously described.

## Etiology

A number of possibilities suggest themselves as causes of the disease described in this paper, and these will be discussed in order.

1. As mentioned in the following section, digestive disturbances may cause in sheep a condition very similar in many of its symptoms to the spasm of our guinea-pigs. Is it not possible that these were originally induced by some similar cause? It is true that at times, especially in the early part of the work, we have had some trouble from improper feeding, notably when we attempted to substitute sugar beets for carrots and cabbage. While, however, inadequate diet may cause scurvy and other effects, we have no reason to believe that it ever produces a condition which could be mistaken for the congenital palsy. Furthermore, palsy never occurs in the descend-

ants of two homozygous normal individuals, even though their feeding and care is in all respects similar to that of the others. In other words, the disease has behaved strictly in accord with the known principles of heredity since it has been under observation, and we have every reason to believe that it has not appeared spontaneously during that time. This would mean then that if the disease was due to nutritive conditions, it must have had its inception in the stock before we received it, or within a very short time thereafter at latest.

The evidence that heritable defects of this sort may be induced by external conditions is very meager at best, Stockard has produced somewhat similar nervous defects in guinea-pigs by the administration of alcohol, but while he claims that these are heritable, he has not, so far as we are aware, shown that any of them are inherited in strict Mendelian fashion as is the defect with which we are concerned. We are therefore led to believe that this character in our stock has not been induced by nutritional or other environmental causes, but that it is due to a factor mutation similar to those which have been studied so thoroughly in domesticated and experimental animals and plants in recent years, and for which there is at present no assignable cause.

2. It might perhaps be assumed that the palsied individuals are due to unfavorable uterine conditions and consequent abnormal fœtal development. The occurrence of "runts" in swine and in other animals which produce large litters show that the uterine conditions are not the same for all the individuals in a litter. Some doubtless have a poorer maternal blood supply than others, or they may be crowded, or twisted into a position unfavorable for growth. That these are not factors in the present case seems demonstrated, however, by the fact that the palsied animals are on the average fully as large and well developed at birth as their litter mates (see Table IV).

3. The necessity of inbreeding the original stock in order that the recessive palsy condition should appear is apparent; but it might be maintained that this inbreeding

in itself was perhaps the cause of the disease. All modern studies on inbreeding, however, seem to strengthen the conclusion that while inbreeding may, by the "concentration" or production of unfavorable character combinations, and particularly by the loss of important physiological factors which are necessary to the well being of the individual, result in lowered vitality and in the appearance of various defects, it is nevertheless a means rather than a cause of bringing these into expression.

Further evidence that the palsy was not produced by the inbreeding as such is furnished by the fact that the lines in which it appeared were no more inbred than many other lines that have been carried on in the laboratory in connection with other problems, but in which no tendency to such a defect has manifested itself. Our whole stock, in fact, of some 2,200 litters and over 5,000 offspring has all descended from not more than 50 original animals, and as has already been stated, there is reason to believe that some of these were related. In order to show the intensity of the inbreeding in some cases, it may be mentioned that in one experiment a male was bred back to his daughters for four successive generations, and with no apparent ill effects. Inbreeding as a predisposing cause may therefore be ruled out.

4. The spasms which form the characteristic reaction of the palsied animals are clearly due to lack of nervous control, especially when voluntary movements of the legs are attempted, and under excitement. It may therefore be that there is some heritable defect of the central nervous system. Examinations which have been made for us by Dr. C. H. Bunting have, however, shown no lesions of the nervous system to which these effects could be attributed.

5. Disturbances of some of the glands which supply internal secretions are known to produce nervous irritability and conditions of spasm and tetany. Particularly is this true of the parathyroid, and some of the symptoms accompanying disturbances of this gland resemble to a certain extent, at least superficially, the con-

ditions in the guinea-pigs. Dr. Bunting is making a study of this phase of the question, and reports that "thus far the only anatomical difference between affected and normal animals of the same litter, that has been noted, has been a definite hypoplasia of the parathyroid tissue in the abnormal animals." He will, however, report more fully later.

For the present, therefore, we must be content with the statement that the congenital palsy is due to a factor mutation, the cause of which is unknown; nor do we understand what its action is on the animal organism to produce the nervous symptoms described.

## Discussion

A number of nervous defects are known in man and the lower animals which have certain points of resemblance to congenital palsy as it occurs in our guinea-pigs, but we have been unable to find any condition which agrees closely enough in details so that the two could be considered identical. A list of some of these follows, with brief mention of resemblances and points of difference.

*Pigeon.—Tumbling* in pigeons appears to be due to lack of nervous control of the muscles and associated to some extent with certain voluntary efforts. This is especially noticeable in Parlor Tumblers, which turn back somersaults when they attempt to fly. The condition is greatly exaggerated by excitement. Further similarities are that the tendency to tumble increases with age, to a certain point at least, and that it behaves in a general way as a recessive to normal flight, though the crossbreds are usually intermediate and there appears to be no sharp segregation in $F_2$. Tumbling, unlike congenital palsy, does not seem to affect the legs particularly, and does not interfere with normal life processes sufficiently to be lethal if the birds are given adequate protection and care.

The condition described by Riddle (1918) as *ataxia* in pigeons would appear to correspond very closely in symptoms to the more pronounced cases in Parlor Tumblers.

There would appear to be also some resemblance to the tumbling and shaking of the Fantail (French *Trembleur*). Riddle states that the "character is, with some irregularities, a Mendelian recessive." His inference that it may have been produced by "reproductive overwork" seems inconclusive.

In connection with experiments on the homing ability of pigeons Hurst (1913) speaks of obtaining "*feeble-minded*" birds, as follows:

> Results show that incompetent or feeble-minded pigeons may be bred from competent or intelligent parents, and it is interesting to find that feeble-mindedness behaves as a recessive character in birds as well as in man.
>
> Fortunately, or unfortunately, it is much more difficult to get offspring from the feeble-minded in Pigeons than in Man.

*Mouse and Rat.*—The well-known "waltzing" of the waltzing mouse is probably of the nature of a nervous disorder, either directly or indirectly. It is a simple Mendelian recessive.

Bonhote (1912) at a meeting of the Zoological Society of London "exhibited living specimens of rats (*Mus rattus*) which he had bred in the course of his experiments, and which showed the 'waltzing' character well known in a variety of the domestic mouse, but which had not hitherto been recorded in rats."

*Rabbit.*—We have in our possession a rabbit which is now several years old, and which has since it was young exhibited characteristic circus movements, or "waltzing," very similar to the activities of the waltzing mouse. This character appeared sporadically and we have been unable to find that it is heritable, even though we have repeatedly bred this male's daughters to their own brothers and back to him. He appears normal in other respects except that one eye seems somewhat distorted, which may have something to do with his behavior. This case differs from the waltzing mouse in that it is probably not heritable, and certainly is not a simple Mendelian recessive.

*Guinea-Pig.*—Some of the various defects in guinea-

pigs described by Stockard and ascribed to the inherited effects of alcohol treatment of the original parents, have symptoms somewhat resembling those of congenital palsy. His descriptions of the symptoms and behavior of his animals are, unfortunately, inadequate for detailed comparison of our cases with his. One point seems certain, however, namely, that while the symptoms exhibited by our animals are relatively constant, he has obtained in his affected lines a great variety of nervous defects and anatomical abnormalities, all of which he attributes to degeneration caused by the alcohol. To mention those relating to nervous disorders, he speaks of the defective animals as being "very shy and excitable" (1912, p. 22), and says further: "it is a point of some interest that all of the young animals that died showed various nervous disturbances, having epileptic-like seizures, and in every case died in a state of convulsion." Again (1913, p. 663) he speaks of an animal which died when one day old, "having been in a constant tremor since its birth; another lived for nine days *but whenever it attempted to walk it was seized with spasmodic contractions;* the third specimen exhibited the same nervous manifestation and was completely eyeless." In a later paper (1916, p. 15) he says that *paralysis agitans* is very common among the $F_1$, $F_2$, and $F_3$ animals, apparently applying this term to some of the symptoms mentioned in earlier papers, and adds that "paralyzed limbs are often observed, the animals being unable to stand or walk."

While some of the above symptoms approximate those of congenital palsy, they seem to partake for the most part either of a general nervous irritability or else of a definite paralysis. Furthermore, while Stockard bases his conclusion that the general defect that produces these various conditions is hereditary on the fact that they continue to appear in his treated lines, but not in the parallel control lines, he has not so far as we are aware, found any tendency for the condition to be inherited in any definite manner or proportions conformable with Mendelian rules.

It is not clear, however, that he has made any systematic matings in an endeavor to ascertain this point.

While we do not mean to imply that in Stockard's experiments the initiation of the various defects and abnormalities he describes may not have been due to the alcohol treatment, it is nevertheless of interest to note that these same defects and abnormalities appear from time to time in our normal stock. We can, in fact, match his conditions—almost case for case for all that he has described—with offspring of our stock that has had the best care we could give it. That our stock is as a whole in no way degenerate is indicated by its prolificacy (average size of 863 litters = 2.71), its low mortality rate, and the fact that our animals are if anything above the general run of guinea-pig stock, as attested by reports from the various hygienic and other laboratories to which our surplus has gone. The point of special interest is that these various abnormalities are entirely independent of the congenital palsy, for they appear no more frequently in the "palsy" stock than elsewhere.

*Goat.*—Hooper (1916) has described a case in goats which has some strong points of resemblance to the behavior in the guinea-pigs, although in the former the conditions are not so severe as to cause the death of the animals. He says:

> There is a peculiar breed of goats raised in central and eastern Tennessee. When suddenly frightened the hind legs become stiff and the animal jumps along until it recovers and trots off normally or if greatly frightened the front legs become stiff also and the goat falls to the ground in a rigid condition. They have received the name of "stiff-legged" or "sensitive" goats.

Experiments were to be begun on the inheritance of the character, but results have not to our knowledge been reported.

*Sheep.*—A condition in sheep with symptoms somewhat resembling those in "palsied" guinea-pigs and even more the goats just mentioned is described by Jones and Arnold (1917). Affected animals are able to walk, but

when excited they run in a stumbling fashion and finally the legs stiffen out and the animal falls on its side. This affection is, however, not heritable, but has been demonstrated by these investigators to be due to nutritional disturbance, caused by a diet consisting too largely of pampas grass.

When there is a liberal amount of grass the actual number of cases is small. After a long continued drought when the fine grass supply is short, the number of sick animals is large. The mortality varies considerably, young sheep seeming to suffer most.

*Man.*—Among the numerous confusing and complex nervous disorders in man there are several with certain similarities to congenital palsy of the guinea-pigs. We have not attempted an exhaustive survey of this field, but list a few of them with remarks on resemblances or dissimilarities. In some cases it is difficult to tell whether the descriptions refer to the same or different affections, the synonymy not being clear. The comparison with congenital palsy is also often uncertain owing to the indefiniteness of the descriptions of symptoms. No attempt at completeness has been made in the matter of references, citations being added merely for giving authority for the statements made.

*Feeble-mindedness* (Davenport, 1911), *epilepsy* (Davenport, 1911; Davenport and Weeks, 1911; Weeks, 1915) and some forms of *insanity* (Davenport, 1911) resemble congenital palsy in being definitely recessive in inheritance, but show no close similarities in other symptoms.

| Similarities | Differences |
|---|---|
| *Paralysis agitans* (Parkinson's disease). (Curschmann, 1915.) | |
| Tremor of muscles. | Appears late in life. |
| Progresses in severity with course of disease. | Constant trembling. |
| | More often in male sex. |
| *Habitual tremor.* (Curschmann, 1915; Dana, 1887.) | |
| Occurs early in life. | Not congenital. |
| Subsides when patient is at rest. | Affects mostly hands and head. |
| Increased by voluntary movements and excitement. | Shallow oscillations. |
| | May disappear. |
| Tendency to be hereditary? (Occurs mostly in neuropathically inclined individuals.) | |

*Familial tremor.* (Curschmann, 1915.)

| | |
|---|---|
| Hereditary. | Not congenital. |
| Usually appears in youth. | Sometimes improvement. |
| Diminishes at rest. | |
| Affects mostly arms and legs. | |
| Progressive in its course. | |
| Treatment powerless. | |

*Tetany.* (Curschmann, 1915.)

| | |
|---|---|
| "Intentional" in some cases. | Tonic spasms. |
| Easily induced by stimuli. | Hands and arms mostly. |
| Legs often attacked. | Spasm duration long. |
| Idiopathic tetany incurable. | Infection indicated. |

*Progressive lenticular degeneration.* (Wilson, 1912; Spiller,[4] 1916.)

| | |
|---|---|
| Bilateral. | Not congenital. |
| Affects both extremities. | Accompanied by cirrhosis of liver? |
| Increase with volitional movement. | Tonic spasticity of face and limbs. |
| Reflexes preserved. | |
| Always fatal. | |
| Familial. | |

*Aplasia axialis extra-corticalis congenita.* (Merzbacher, 1908; Batten and Wilkinson, 1914.)

| | |
|---|---|
| Congenital or in first three months. | Affects chiefly males. |
| Hereditary. | Slowly if at all progressive. |
| | Symptoms constant. |
| | Not so fatal. |

*Paramyotonia congenita.* (Eulenberg, 1886.)

| | |
|---|---|
| Congenital. | Tonic spasms. |
| Hereditary. | Not always bilateral. |
| | Last for hours. |
| | *Apparently dominant.* |

It is clear that none of the above-mentioned conditions can be considered as identical with congenital palsy. The most common similarity is that several of them are known to be recessive in inheritance, but they all differ in other symptoms. Congenital palsy differs from any of the other conditions in being definitely congenital and running a brief course terminating in death at an early age.

In conclusion it may be pointed out that while the data may never be sufficiently complete in man, it may be pos-

[4] According to Spiller the conditions attributed to disease of the lenticular nucleus are numerous, including the pseudo-sclerosis of Westphall and Strümpell, Huntington's chorea, Parkinson's disease, and a number of others.

sible in animals where breeding experiments can be conducted, to use the inheritance method for separating nervous diseases in which the symptoms are so similar as to be confusing, or even identical. For example, let us suppose that a recessive neurosis similar to congenital palsy should appear in another line of guinea-pigs. If animals heterozygous for it were mated to heterozygous individuals of our stock and they produced affected offspring in a one-to-three ratio, it would be good evidence that we were dealing with the same heritable trait in both strains. If, however, the disease in the new line was genetically different, a different ratio of offspring would be expected, presumably nine normal to seven neurotic individuals, assuming that there was no linkage of the two genes concerned. It is possible that even in man when the family histories are sufficiently complete the method of genetic analysis may help in the differentiation of neuroses characterized by symptoms which are confusingly similar.

## Summary

1. A definite neurosis appeared in our guinea-pig stock in 1914, characterized by clonic spasms, particularly of the legs. When in a spasm the animals lie on their sides in a helpless condition. This state is induced by various stimuli, but especially by those of a sharp auditory nature, and also by attempted volitional movements of the legs.

2. The affected animals are fully up to average weight when born, and appear to be normal in all other respects. While different individuals vary with respect to the intensity of the symptoms at birth, they are always easily distinguished from normal young, and in all cases the disease runs a short progressive course, terminating in death within about two weeks at most.

3. This defect, which we have called congenital palsy, is definitely heritable. It is a simple Mendelian recessive, and normal and affected offspring are produced by two

heterozygous parents in the ratio of three normal to one affected.

4. It has been shown that heterozygous animals mated to normals produce offspring of the same classes as themselves in equal numbers. Furthermore, it has been proven that homozygous dominants can be extracted from heterozygous parents and that they show no more tendency to transmit the disease than individuals of normal unrelated stock.

5. Heterozygous animals are entirely normal in their reaction and can be told from the homozygous only by breeding tests.

6. A survey of the literature relating to nervous defects in man and other animals does not reveal any condition corresponding exactly to congenital palsy. Some of the conditions in pigeons, rodents and in man are similar in that they are recessive in inheritance.

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# ANIMAL LIFE AND SEWAGE IN THE GENESEE RIVER, NEW YORK*

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It is a hopeful sign of permanent improvement in our rivers and streams when commonwealths and municipalities turn their attention to the condition of these waters and provide means for their purification where they have previously been contaminated by sewage, refuse, or chemicals.

It has been known to biologists for many years that sewage and chemicals were inimical to the life inhabiting these waters, but political bodies have been slow to realize or to admit that the pouring of millions of gallons of crude sewage had any effect on the animal life living in such waters. It is even probable in some cases that those in authority cared little about the effect of such contamination, if it provided an easy and economical method of disposing of the sewage. The damage to fish and other aquatic life has not been realized by the engineers in charge of such work and hence this class of scientific men has not protested or sought a better method, at least not until recent years. The work of the various conservation commissions of the several states, as well as the efforts of natural history societies, universities, and private individuals, have brought into prominence the danger from stream pollution and have awakened widespread interest in this important subject.[1]

In Illinois, careful studies are in progress by the Nat-

* Contribution from the Museum of Natural History, University of Illinois.

[1] See in this connection, Henry B. Ward, "The Elimination of Stream Pollution in New York State," *Trans. Amer. Fisheries Soc.*, XLVIII, pp. 1–25, 1918.

ural History Survey under the direction of Dr. S. A. Forbes, for the purpose of ascertaining the effect in the Illinois River of the large volume of polluted water from the Chicago Drainage Canal, into which all of the sewage of the city of Chicago is discharged.[2] In other places, studies of a similar character are being carried on.

In New York State, the Genesee River, at Rochester, has afforded a striking example of stream pollution, of the effect of this pollution on the animal life in the river, and of the final return of this life after the amount of pollution was notably reduced. It has been the writer's good fortune to visit Rochester every two or three years (sometimes oftener) and to be able to study the condition of the Genesee River during a period of nearly thirty years. Collections were made before, during, and after pollution, permitting comparisons to be made of the life in the river during these several periods of varying conditions.

The animal life in a body of water has been little used as an indicator of the degree of pollution. Fish, especially young fish, have been used and are good indicators because they cannot live in water polluted to any large degree. The relative resistance of different species of fish has been well shown by Shelford in a recent paper.[3] The writer is convinced that mollusks are also good indicators of degrees of pollution. The intimate relation of fish to the propagation of river mussels (Unionidæ), so largely used in the manufacture of pearl buttons, is also seriously affected by stream pollution.

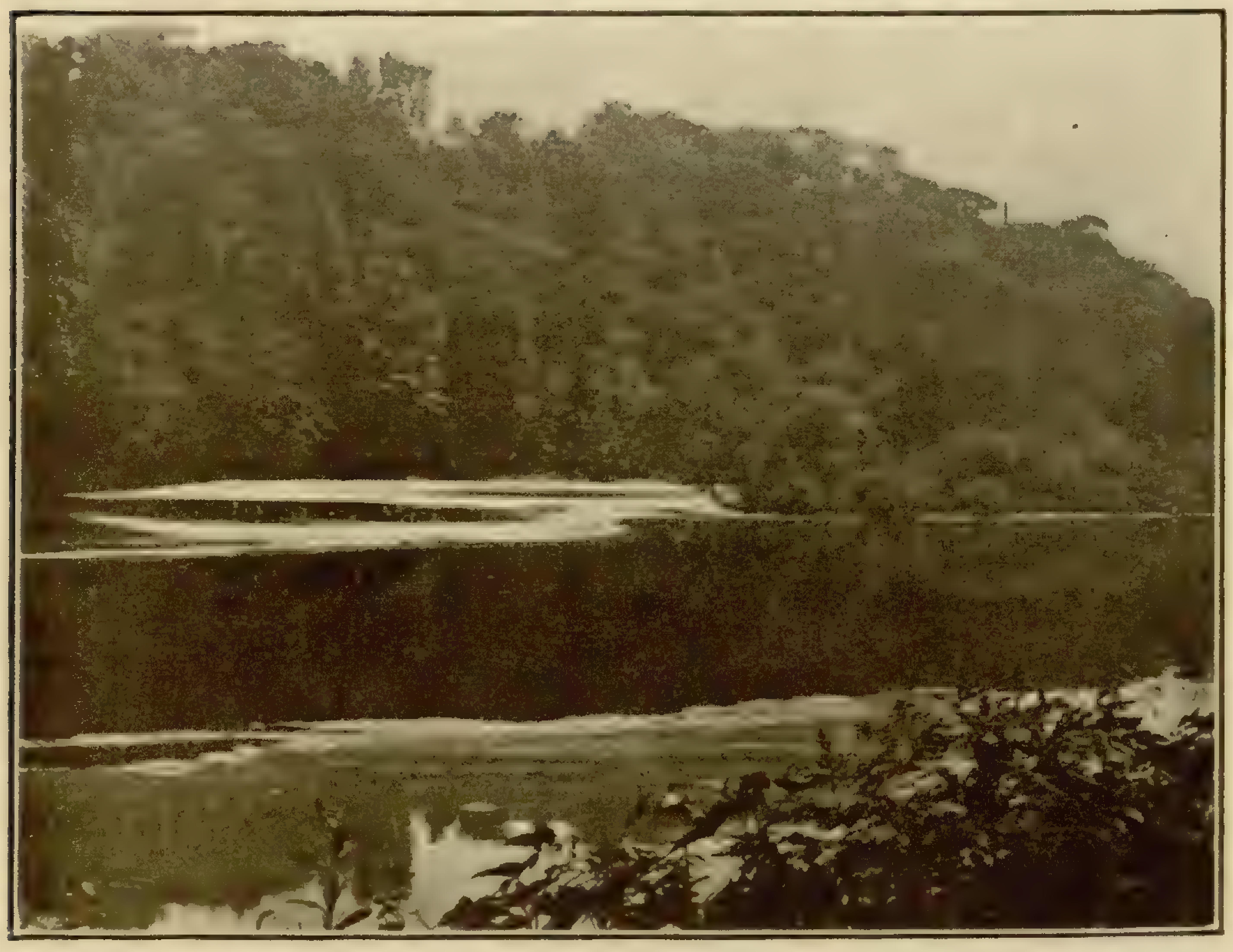
The polluted portion of the Genesee River studied (which was also the place of maximum pollution) lies below the lower falls (Driving Park Avenue bridge), a large sewer discharging some distance below these falls just above the spot known locally as Brewer's landing, near Norton Street, on the east side of the river. Several

[2] See Forbes and Richardson, "Some Recent Changes in Illinois Biology," Illinois Natural History Survey, *Bulletin*, XIII, pp. 139–156, 1919.

[3] *Bull.* XIII., Ill. Nat. Hist. Surv., pp. 25–42, 1918.

other sewers emptied into the river at the north end of Maplewood Park on the west side of the river. The stream for several miles below these points resembled thick, dirty, greasy dish water, a heavy scum covering the surface of the water as well as the shore and any objects in the water.

At the time of maximum discharge, sewer outlets also entered the river both above and below the falls and



The photograph was taken in the summer of 1907. The white streaks in the background are from the trunk line sewer on the east side of the river near Norton Street. The light streak in the foreground is the sewage from the outlet on the west side of the river near the lower falls. A small island on the right side of the picture hides a part of the sewage in the water on the east side of the river. This place is six miles up the river from the mouth.

many manufactories also contaminated the water by discharging chemicals and refuse into the river. At the present time there are no sewers entering the river above the falls. There are two sewer outlets at the northern end of Maplewood Park, one from the residence section west of the river (Dewey Avenue section) and one from the Eastman plant. Sewage is also discharged into the

river at Charlotte near the mouth of the river. This, however, does not enter into the present discussion. It has not been possible to ascertain the amount of sewage entering the river during the period of maximum discharge, but at the present time the approximate quantity of sewage discharged is one-eighth cubic foot per second (data from Rochester engineering department). This is a comparatively small amount which apparently has little or no effect on the animal life in the river. On the contrary, it may provide food for some organisms.

Sewage was turned into the Genesee River about the year 1820. Collections of molluscan life were made in July, 1897, and nine species were obtained, as noted below:

| | |
|---|---|
| *Musculium transversum* | *Physa gyrina* |
| *Musculium partumeium* | *Physa sayii* |
| *Bythinia tentaculata* | *Physa heterostropha* |
| *Galba catascopium* | *Galba caperata* (rare) |
| *Planorbis trivolvis* | |

Previously, in 1892–1895, collections had been made which included about the same species as noted above. Individuals were notably abundant, thickly covering the rocks and the shore. At the time the above mollusks were collected it was noted that the sewage was increasing in volume and pollution was becoming more noticeable. It was predicted at this time that in a few years the fauna would be exterminated by the foulness of the water.

The river was visited and examined in 1898, 1900, 1901, 1904–1907, 1908, 1910, 1913, 1915–1917, and 1919.[4] Each year it was noted that the pollution of the water was rapidly increasing. In 1907, the water-breathing mollusks, *Musculium* and *Bythinia,* had succumbed and none could be found. The air-breathers, *Galba, Planorbis, Physa,* still held out, though reduced in number of indi-

[4] See the following papers by the writer: "The Molluscan Fauna of Western New York," *Trans. Acad. Sci. St. Louis,* VIII, pp. 71–94, 1898. "The Molluscan Fauna of the Genesee River," AMER. NAT., XXXV, pp. 659–664, 1901.

viduals. An examination made in 1910 failed to discover a single living mollusk of any species. Apparently, the water had reached such a state of concentrated pollution that even the air-breathing mollusks, which normally come to the surface to take in free air, could not adapt themselves to this unfavorable environment and were either killed or compelled to migrate down the river to a point where the pollution was not so great, a distance of several miles. During the following two or three years the river was visited but no mollusks could be found.

On March 17, 1917, a large part of the city's sewage was diverted to the Irondequoit sewage disposal plant located on the shore of Lake Ontario near the Durand-Eastman Park. Here an average of 32 million gallons of sewage are treated daily, and the treated sewage discharged into Lake Ontario at a distance of 7,000 feet from shore in water 50 deep (vide city engineer's statement). It may easily be seen that when this large amount of sewage, untreated, was discharged into the Genesee River, it could not but render the water totally unfit for animal life and a menace even to the inhabitants who visited the beautiful parks bordering both sides of the river.

The result of the diminution of the amount of sewage discharged into the river has been that the fauna has returned and has rapidly taken possession of the favorable environments which were in use previous to the maximum period of pollution. Collections made in September, 1919, contained the six species noted below:

*Musculium transversum* *Bythinia tentaculata*
*Planorbis trivolvis* *Galba catascopium*
*Physa integra*
*Physa oneida* (previously reported as *heterostropha*)

It will be noted that practically the same species returned to the Maplewood Park section of the river that lived here before the polluted water exterminated the fauna, indicating, probably, that they migrated up the river from the less affected water below.

Few data are at hand indicating how far the polluted water must flow before it can purify itself enough to become favorable for animal life. In the Illinois River, life is being affected by the Chicago sewage at Peoria, a distance of 110 miles from the source of infection. A recent study of the Salt Fork of the Vermilion River, into which the sewage of Champaign and Urbana is discharged, indicated that the polluted water was inimical to molluscan life for a distance of 14 miles in which no living mollusks were found, and one must pass down the stream for a distance of nearly twenty miles before a normal mussel fauna can be found.

In the Allegheny River, Ortmann found that whole stretches of the stream and some of its tributaries had been made into a desert by pollution, principally in the form of chemicals from the numerous mines situated in this part of the State. Ortmann remarks that "with regard to the animal life in our rivers, sewage does not seem to be harmful; on the contrary, certain forms (fishes, crawfishes, mussels) seem to thrive on it" (p. 97). This is probably true in a case where water is but slightly contaminated; but in streams where pollution by sewage is greatly concentrated (a condition reached sooner or later in all streams used for sewage disposal) it is certainly inimical to the forms of life mentioned. It is very true that a stream polluted by chemicals soon becomes destitute of the larger forms of animal life (if, indeed, not all life) and in such waters the return of life will be very slow and in many cases it may be impossible for life to return on account of the chemicals which cover the bottom and shores.

In the case of the Genesee River, we have a striking example of the history of a polluted stream and its effect on the animal life. Previous to the discharge of sewage into the stream there was a varied molluscan fauna very numerous in individuals. In the course of eleven years the gill-bearing mollusks were forced out and after a lapse of fourteen years all molluscan life ceased to live

in this portion of the river. Seven years later, the greater amount of sewage was diverted to another outlet. Two years after this diminution of pollution we find that the molluscan fauna has returned in as great number of individuals as were found there before pollution began. In other words, it required but two years (possibly less, as the river was not examined in 1918, one year after the conditions changed) for the river to become pure enough to provide a favorable environment for molluscan life. It has been reported that the sturgeon is again resorting to the lower portion of the river for spawning purposes, after an absence of several years, due to the heavy pollution of the water. The rapid return to a favorable condition is partly due to the lower falls in the river which abundantly aerates the water before it is mixed with the small amount of sewage now flowing into the stream.

It may be affirmed without successful contradiction that wherever sewage pollution occurs, sooner or later the animal life will be affected, and finally driven out. As this condition seriously concerns our food and game fishes, which form so large a part of the meat food of our population, it is a situation that demands immediate attention and early remedy. That the fauna recovers so quickly after pollution ceases is a matter of great interest and satisfaction, showing what favorable returns may be expected when these matters are taken up in all earnestness by municipalities and commonwealths.

### Pollution in the Genesee River

Since writing the above account of the effect of sewage pollution on the molluscan life of the river, Mr. John F. Skinner, principal assistant engineer of the Rochester Department of Engineering, who has been connected with this department for upwards of twenty-eight years, has kindly read the paper and has indicated several inaccuracies in the historical matter, besides adding much information of value concerning the sewage disposal of the city. The following data are all supplied by Mr. Skinner:

Rochester was settled in 1812, incorporated as a village in 1817, and as a city in 1834. The first sewers were built about 1820. All of the city west of the river and roughly everything within a mile east drained into the river. A sewer 4 by 6 feet in diameter was in operation in 1863. In 1896 nine main outfalls were in operation, five on the west side and four on the east side. In 1897 the west side trunk sewer nine feet in diameter was put in operation. Four of these sewer outfalls are below the lower falls, two being above the point at which the molluscan studies were made.

Fourteen stormwater sewers overflow into the river above the lower falls. Refuse and waste matter, both liquid and solid, enter the stream from a tannery, gas works, breweries, garbage disposal plants and some other manufacturing plants. The breweries do not now contaminate the water as formerly. This additional pollution is sometimes more harmful to animal life than the sewage itself. In March, 1917, the main (Irondequoit) sewage disposal plant was put in operation on the shore of Lake Ontario. The outfall to this plant intercepts the dry weather flow of all of the sewer outlets mentioned above, except the Lake Avenue or Dewey Avenue outlet on the west side about 6,000 feet below the lower falls. There is also a large outlet further north, down the river, from the Eastman Kodak plant and adjacent territory. The overflows from the east and west side trunk sewers enter below the lower falls, as will also that from the Lake Avenue sewer after the Maplewood plant is completed. The clarified effluent of the last mentioned plant will also enter the river, but the major portion of the solids (contained in the sludge) will be pumped across the river to the east side interceptor.

In a report issued in 1913,[5] Mr. George C. Whipple, consulting engineer, has published much valuable information relating to the effect of the sewage pollution on

[5] "Report on the Sewage Disposal System of Rochester, N. Y.," by Edwin A. Fisher, city engineer. See pages 179–200.

the river and on some of the animal and vegetal life. This study was made in 1912 when the pollution was at its maximum and during the period when molluscan life had disappeared from the upper part of the river below the lower falls. The dissolved oxygen in the lower river, below the trunk line sewer, in July and August when the temperature was high and the water low, varied from 5 to 41 per cent. of saturation. The water at the bottom of the river almost always contained less oxygen than that at the surface. This condition prevailed to within a short distance of the mouth of the river when the reverse was true, this change being due to the backflow of the well-oxygenated water from Lake Ontario. Near the east side trunk sewer, which is about half a mile below the lower falls, the percentage of saturation varied from 5 to 60 between July 1 and August 13. On August 13, the percentage of saturation between the east side trunk sewer and a point two and a half miles from the lake (a distance of about three miles) did not exceed five per cent. This area includes the shores examined for the Mollusca. The percentage of dissolved oxygen saturation was usually higher at the surface than at the bottom of the river, the heavier parts of the sewage falling to the bottom and forming sludge banks. The percentage of dissolved oxygen also did not vary directly with the amount of evident pollution, for on a day in July when the most disagreeable conditions existed for a mile and a half below the east side outlet the dissolved oxygen at the surface varied from 40 to 70 per cent.

A study of the plankton of the river indicated that near the source of pollution, 5½ miles above the mouth of the river, there were on the average in July and August, 1,650,000 bacteria, 156 algæ, 209 Protozoa, and 57 Crustacea and Rotifers per cubic centimeter. At the mouth of the river the figures for these organisms per c.c. stood as follows: 67,000; 363; 77; 233. It is unfortunate that no discrimination was made between the foul water algæ and protozoa and those normally inhabiting pure water,

which would have made a difference in the number from the standpoint of pollution. This has been done by Forbes and Richardson in their studies of the Illinois River pollution.[6]

A comparison of the report made by Engineer E. Kuichling, Feb. 1, 1907 (1913 report, pp. 5–42) with that of Mr. Whipple made in 1912 shows in a striking manner the rapid increase of polluted conditions, the former author describing conditions as not very bad (pp. 10–11) while the latter author, five years later, describes the conditions as very bad (p. 182). It was between these dates, 1907 and 1912, that the molluscan fauna disappeared and it is apparent that the distinct increase in toxicity is indicated from these several angles of vision.

It should be stated in connection with the ill effects of sewage pollution that it affects the population in an indirect manner not usually recognized by sanitary engineers who have not interested themselves in the problem of fish culture. Such places as the six miles of shallow shore bordering the Genesee River are the breeding and feeding ground of such valuable food and game fish as the sturgeon, black bass, sunfish, suckers, bullheads, pickerel, pike, etc., and the young of these and other fish spend a large part of their life in this kind of a habitat, to later migrate into the open lake.

[6] "Studies on the Biology of the Upper Illinois River," *Bull. Illinois State Laboratory of Natural History*, IX, pp. 481–574, 1913.

# ALTERNATIVE EXPLANATIONS FOR EXCEPTIONAL COLOR CLASSES IN DOVES AND CANARIES

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DOVES and canaries have been shown to possess certain color factors which are sex-linked in inheritance. The behavior of these factors leads one to the conclusion that, unlike *Drosophila,* cats and man, the female and not the male is the homozygous sex. In this respect they resemble *Abraxas,* poultry and the domestic pigeon.

In both doves and canaries, however, there occur exceptional color classes in certain matings where the sex-linkage of the factors in question manifests itself. To explain the appearance of these exceptional color classes, Sturtevant (1912) (in canaries) and Bridges (1913) (in doves) have suggested that the principle of *partial sex-linkage* is involved.

Later, Bridges (1916), in discussing the phenomena of *non-disjunction* in *Drosophila,* has reviewed briefly other forms to which he considers non-disjunction may apply and among these mentions doves and canaries as follows:

> (Doves—p. 157) Exceptions to the inheritance of blond and the dark types of pigeons have been explained as partial sex-linkage (Bridges, 1913) but non-disjunction offers an alternative hypothesis which seems plausible.
>
> (Canaries—p. 158) Exceptions to sex-linkage in the inheritance of pink versus black eye colors have been reported (Durham and Marryatt, 1908). These exceptions are explainable by non-disjunction or by partial sex-linkage.

In this paper an attempt will be made to show that the hypothesis of partial sex-linkage and of non-disjunction expect certain results from the crosses made, which have not been reported and in the case of non-disjunction might, in addition, be fairly considered as involving sterility of certain color classes—a phenomenon not yet reported in any of the forms in question.

A further effort will be made to explain the observed facts on an hypothesis of *factorial change* which involves neither a break

in sex-linkage nor non-disjunction, and which expects no unusual sterility nor the appearance within the crosses made, of other unrecorded exceptions to the normal relation between phenotypic color classes.

We may take up in order the three hypotheses of partial sex linkage, non-disjunction and factorial change and may compare them on the basis of the experimental results obtained.

## Partial Sex-linkage

1. *Doves.*—The normal result obtained when white male and colored female ring doves are crossed, is colored males and white females. This may be explained as follows: Let W equal a factor for the production of colored plumage and w, a factor allelomorphic to it for the production of white plumage. The male is FFMM, the female FFMm in formula. W is linked with M in inheritance.

| | White Male wwFFMM | Colored Female WwFFMm |
|---|---|---|
| Forming gametes | wFM | WFM and wFm |
| Zygotes obtained: | WwFFMM = colored males | |
| | wwFFMm = white females | |

In addition, however, exceptional colored females are produced infrequently and have been recorded by Staples-Browne (1912), and by Strong (1912). These exceptional colored females have been acounted for by Bridges (1913), as follows:

If in the female the sex-differentiating factor and the factor for plumage color are placed close enough together in the same chromosome to be linked, but not so close that linkage is complete "crossing-over" would cause the two factors which entered in the same member of the homologous pair of chromosomes to lie in different members and hence to segregate to different gametes.

If this occurred we should have the following condition:

| | White Male wwFFMM | × | Colored Female WwFFMm | |
|---|---|---|---|---|
| Forming gametes | wFM | | WFM, wFm | commonly |
| | | | wFM, WFm | exceptionally |
| Zygotes formed: | WwFFMM colored male | | | commonly |
| | wwFFMm white female | | | commonly |
| | WwFFMm colored female | | | exceptionally |
| | wwFFMM white male | | | exceptionally |

This would account for the exceptional colored females, reported from this cross by Staples-Browne and by Strong. It also expects an additional exceptional class—namely white males, and these, though they should occur as frequently as do the colored females, are conspicuous by their absence.

Further than this the hypothesis as just outlined supposes that "crossing over" occurs in the heterozygous sex, between chromosomes which correspond to the X and Y chromosomes of *Drosophila*. This condition has not been observed in *Drosophila* or in forms where a similar opportunity exists and it must therefore, be considered as entirely hypothetical and contrary to such evidence as the most extensively investigated forms have given.[1]

2. *Canaries.*—In this form, the sex-linked inheritance of the factor for dark-eyed color (P) having as an allelomorph pink eye color (p) has been demonstrated by Durham and Marryatt (1908) and reviewed by Sturtevant (1912). Here, however, as in doves there is an unexpected color class which makes its appearance. The exceptional individuals are dark-eyed females which occur in a cross between pink-eyed males and dark-eyed females where only dark-eyed males and pink-eyed females are expected.

Sturtevant, in reviewing the case and in attempting to explain it as the result of partial sex linkage says (p. 570):

This hypothesis could be easily tested. If it is correct, then the cross just discussed should, if large enough numbers be reared, produce as many pink-eyed males as black females." The occurrence of such pink-eyed males has not been reported although it seems almost certain that their appearance in this cross would have been observed and mentioned by breeders did they occur even very rarely.

We may, therefore, say that should the missing color classes appear in the dove and canary matings as predicted by the hypothesis of partial sex linkage, that hypothesis would have a

[1] Cole and Kelley (1919) have studied the linkage relations between two sex-linked factors in the domestic pigeon and have on the basis of considerable data, come to the conclusion that "crossing over" occurs in the male but *not* in the female. This result seriously invalidates partial sex-linkage as a possible explanation for the exceptionally colored females in doves or dark-eyed females in canaries. Furthermore, Goodale (1917) has shown that "crossing over" has occurred in the male and not in the female of the domestic fowl—a point which also has a direct bearing on the work with doves and canaries.

stronger claim to recognition as the correct one for explaining the observed phenomena. Until such time, however, the explanations of the observed exceptions by an hypothesis which requires the appearance of an approximately equal number of exceptions in the same cross, without any evidence that such exceptions exist can not be considered as satisfactory. That this is the case for doves has been suggested by Bridges in his more recent (1916) paper on non-disjunction as already quoted.

### Non-disjunction

In 1916 Bridges, in giving the data on which rests most of the experimental proof of the existence of non-disjunction in *Drosophila,* suggested that the exceptions to sex-linked inheritance in doves and canaries, might be the result of non-disjunction of the sex chromosome.

If, however, the matings producing such exceptional individuals are analyzed on the basis of non-disjunction, certain discrepancies between expectations and actual results become evident. These discrepancies suggest some fundamental difficulties in applying the hypothesis of non-disjunction to the cases in question. Thus if we assume as does Bridges that the sex formula in both doves and canaries is FFMM in the male and FFMm in the female we may represent the crosses made as follows: Theoretically there may be either (*a*) no non-disjunction, or (*b*) non-disjunction in the male, or (*c*) non-disjunction in the female. In each mating we shall consider the three possibilities:

1. In doves:

*White Male × Colored Female*

| | White Male<br>wwFFMM | × | Colored Female<br>WwFFMm |
|---|---|---|---|
| (*a*) If *no non-disjunction;* forms gametes | wFM | | WFM<br>wFm |
| (*b*) If *non-disjunction in the male;* forms gametes | wwFFMM<br>and — | | WFM<br>wFm |
| (*c*) If *non-disjunction in the female;* forms gametes: | wFM | | WwFFMm<br>and — |

In the three cases the zygotes formed will be as follows:

(*a*) *If no non-disjunction:*

(1) WwFFMM = colored males
(2) wwFFMm = white females

This is the usual result obtained with, however, the addition of exceptional dark females which we are trying to explain.

(*b*) If non-disjunction in the male: forms the following zygotic classes:

(1) WwwFFFMMM = males? Colored? Probably die.
(2) WFM — = colored females? Sterile?
(3) wwwFFFMMm = white males (transmitting non-disjunction).
(4) wFm — = whites? Probably die.

It should be noted that non-disjunction in the male of *Drosophila* does not occur. Yet in pigeons the *male* is presumably the "homozygous" sex and this makes its chromosome condition in respect to sex more closely analogous to the *female Drosophila* in which primary non-disjunction *does* occur. The classes b(1) and b(4) we may fairly suppose, fail to survive. The triple X condition of form b(1) is fatal in *Drosophila* as is the absence of both X and Y seen in class b(4). The two classes b(2) and b(3) are however, real difficulties. The sterility of class b(2), the exceptional colored females has never been reported, as would undoubtedly have been the case, did it exist—just as their appearance alone has excited comment and interest. Class b(3), moreover, would certainly be *white males* and as we have already seen there is no record of any such animals appearing in this cross. Since these white males are the means of transmitting the tendency for non-disjunction to further generations, their presence is necessary for the occurrence of secondary non-disjunction and their absence is a serious handicap to the acceptance of the hypothesis in this material.

(*c*) Non-disjunction in the female:

(1) WwwFFFMMm = colored males (transmitting non-disjunction).
(2) wFM — = white females? Sterile?

It will be seen that the exceptional colored females are neither expected nor explained by this type of non-disjunction. Unless, therefore, we are to assume that non-disjunction in doves is, in almost all its fundamentals, different from the same process in *Drosophila*, producing qualitatively different results, we must agree that it fails to meet the experimental facts. If we do suppose that it differs fundamentally, it may fairly be claimed that no evidence of a conclusive nature either cytological or genetic exists to lead us to say that non-disjunction is in any way involved.

Although the cross just considered is the one chiefly cited, it is of interest to attempt to apply the three possibilities in question to the reciprocal cross namely colored male × white female—as follows:

| | Colored Male WWFFMM | × | White Female wwFFMm |
|---|---|---|---|
| (*a*) If no non-disjunction; forms gametes: | WFM | | wFM and wFm |
| (*b*) If non-disjunction in the male, forms gametes: | WWFFMM and — | | wFM and wFm |
| (*c*) If non-disjunction in the female, forms gametes: | WFM | | wwFFMm and — |

(*a*) No non-disjunction. A normal mating of this type gives two classes of offspring as follows:

(1) WwFFMM = colored males.
(2) WwFFMm = colored females.

No exceptions have been recorded.

(*b*) Non-disjunction in the male: would expect four types of zygotes as follows:

(1) WWwFFFMMM = colored males? Probably die.
(2) WWwFFFMMm = colored males—transmitting non-disjunction.
(3) wFM — = white females? Sterile?
(4) wFM — = whites? Probably die.

Did class b(3), sterile white females occur, their appearance would undoubtedly have been noted and recorded. It should further be noted that non-disjunction, namely in the male, is the only type able to account for the appearance of exceptional colored females in the cross reciprocal to that just considered. In this cross, however, this type of non-disjunction expects a color class which has not been recorded in matings of supposedly homozygous colored males.

(*c*) Non-disjunction in the female:

(1) WwwFFFMMm = colored males transmitting non-disjunction.
(2) WFM — = colored females? Sterile?

Here, non-disjunction gives no exceptions save the occurrence of an occasional sterile colored female. This would undoubtedly

be able to escape detection by breeders unless the most careful individual records were kept. However, non-disjunction in the female fails entirely to account for the occurrence of the observed exceptions in the reciprocal cross and may for that reason be disregarded.

Instead, therefore, of increasing the probability that non-disjunction is involved in the production of the exceptions noted, a consideration of the above cross shows that it either fails to account for the exceptions which *do* occur or else expects additional color classes which have not been observed.

2. *Canaries:*

Here the conditions differ but slightly from those already described for doves. The factors involved are P, dark eye color epistatic to p, pink eye color. P is commonly considered as sex linked. The possibilities of non-disjunction remain the same as in doves and may, therefore, be taken up under parallel headings.

*Cross of Pink-eyed Male × Dark-eyed Female.*

| | Pink-eyed Male ppFFMM | × | Dark-eyed female PpFFMm |
|---|---|---|---|
| (*a*) If no non-disjunction; forming gametes | pFM | | PFM and pFm |
| (*b*) If non-disjunction in the male: forming gametes | ppFFMM and — | | PFM and pFm |
| (*c*) If non-disjunction in the female: forming gametes | pFM | | PpFFMm and — |

The zygotes formed by these three processes would, in order, be as follows:

(*a*) No non-disjunction:

(1) PpFFMM = dark-eyed males.
(2) ppFFMm = pink-eyed females.

It is in this cross that exceptional dark-eyed females sometimes occur.

(*b*) Non-disjunction in the male:

(1) PppFFFMMM = probably dies.
(2) PFM — = dark-eyed females? Sterile?
(3) pppFFFMMm = pink-eyed males transmitting non-disjunction.
(4) pFm — = probably dies.

Here, as Durham and Marryatt who reviewed the case have stated, although canary breeders have long noticed the occur-

rence of dark-eyed females, *no mention is made of their sterility nor of the occurrence of Class b(3), pink-eyed males, although the latter should have appeared with approximately equal frequency.* When one considers the amount of canary breeding which has been done, and is still being continued and the fact that breeders have long recognized the exceptional dark females, the continued absence of the expected pink-eyed males becomes a real objection to the acceptance of any hypothesis which calls for their appearance.

(*c*) Non-disjunction in the female:

(1) PppFFFMMm = dark-eyed males transmitting non-disjunction.
(2) pFm — = pink-eyed females? Sterile?

It will be noted that this type of non-disjunction fails, as it did in doves, to account for the observed exceptional dark-eyed females.

Further if we now consider the reciprocal cross of dark-eyed male × pink-eyed female, we shall find that the only type (*b*) of non-disjunction which is able to account for the exceptional color class above recorded demands a type of result as yet not reported.

| | Dark-eyed Male PPFFMM | × | Pink-eyed Female |
|---|---|---|---|
| (*a*) If no non-disjunction; forms gametes | PFM | | ppFFMm<br>pFM and pFm |
| (*b*) If non-disjunction in the male; forms gametes | PPFFMM and<br>— | | pFM and<br>pFM |
| (*c*) If non-disjunction in the female; forms gametes | PFM | | ppFFMm and<br>— |

The following classes of zygotes will be expected:

(*a*) If no non-disjunction:

(1) PpFFMM = dark-eyed males.
(2) PpFFMm = dark-eyed females.

No exceptions have been recorded.

(*b*) If non-disjunction in the male:

(1) PPpFFFMMM = dark-eyed? Probably dies.
(2) PPpFFFMMm = dark-eyed males transmitting non-disjunction.
(3) pFM — = pink-eyed females? Sterile?
(4) pFm — = probably dies.

Here it will be observed that pink-eyed females, probably sterile, should be produced as frequently as were the exceptional dark females in the reciprocal cross. We have no evidence that this is the case.

(*c*) If non-disjunction in the female:

(1) PppFFFMMm = dark-eyed males transmitting non-disjunction.
(2) PFM — = dark-eyed females? Sterile?

Here, as in doves, the result would possibly be masked because no unusual color type is expected. Since, however, this form of non-disjunction would fail to account for the dark females in the cross of pink-eyed male × dark female it may be disregarded.

To sum up, we may say that non-disjunction is able to explain part of the observed facts but expects sterility and other exceptional color classes in crosses where they have not been found. If then, an explanation can be found which expects, in the crosses made, the observed color classes and none other, it should in the absence of stronger evidence for non-disjunction, be considered to be fully as likely an explanation of the phenomena observed.[2]

## Factorial Change

1. *In Doves.*—In a paper now in press, I have attempted to show that the occurrence of exceptional color classes in cats (other than tortoise-shell males) which have been variously interpreted as due to partial sex-linkage or to the action of modifying factors, may be satisfactorily explained by a process of factorial change. Thus if in cats in some of the gametes of certain unusual individuals the factor Y for the restriction of black pigment from the coat, appeared in its hypostatic and allelomorphic form y, the exceptional color classes would be accounted for. If a somewhat similar process occurred in certain rare individuals in doves, between factors W and w, but in the reverse direction, namely, from w white to W colored, we should have an explanation for the exceptions observed.

If, then certain white male doves formula wwFFMM formed

[2] Cole and Kelley (*loc. cit.*) believe that the exceptional colored females in crosses between white male and colored female doves are simply mistakes in observation or records. Because of the fact that they were obtained by two entirely independent investigators and because a similar exception is found in the case of canaries where even more extensive evidence exists, it is believed that the case demands explanation and cannot be merely disregarded as Cole and Kelley imply.

among their gametes some that were WFM instead of wFM the following result would be obtained in a cross between one of them and a colored female.

| | White Male wwFFMM | × | Colored Female WwFFMm |
|---|---|---|---|
| Forming gametes: | wFM commonly | | WFM |
| | WFM exceptionally | | wFm |

Zygotes expected: (1) WwFFMM = colored males (commonly)
(2) wwFFMm = white females (commonly)
(3) WWFFMM = colored males (exceptionally)
(4) WwFFMm = colored females (exceptionally)

Of the two exceptional zygotic classes (3) and (4) only the latter represents a distinguishable phenotypic difference. The other (3) would be merely individuals homozygous for the factor W and therefore indistinguishable, except by proper breeding tests, from the common heterozygous class (1). Such tests have not been reported on as yet by investigators in whose stock, class (4) individuals have appeared. The point to be emphasized, however, is that the presence of homozygous colored males might very easily escape notice unless sufficient numbers of young from a critical cross were raised. It should further be noted that no sterility is expected nor has any been recorded.

If a similar change occurred at rare intervals in certain *white females* as we have a right to expect it possibly would, we should have *no phenotypically aberrant or unexpected color classes formed* in crosses between such females and colored males. We should however, obtain infrequently as a result of this process *homozygous* instead of heterozygous colored males and peculiarly constituted colored females as follows:

| | Colored Male WWFFMM | × | White Female wwFFMm |
|---|---|---|---|
| Forming gametes: | WFM | | wFM, wFm commonly |
| | | | WFM, WFm exceptionally |

Zygotes expected: (1) WwFFMM = colored males, (2) WwFFMm = colored females — commonly.
(3) WWFFMM = colored males, (4) WWFFMm = colored females — exceptionally.

Here again only careful individual breeding tests would be expected to reveal the presence of the exceptional homozygous

colored individuals of classes (3) and (4) and no sterility above the ordinary would be expected.

It is also interesting to note that theoretically the colored females of class (4) would when crossed with ordinary white males yield *colored females* of the normal type. Thus:

| | White Male wwFFMM | × | Colored Female Class (4) WWFFMm |
|---|---|---|---|
| Forming gametes: | wFM | | WFM<br>WFm |

Zygotes expected: (1) WwFFMM = colored males.
(2) WwFFMm = colored females.

It may be objected that changes from a hypostatic factor to its epistatic allelomorph are not frequent. This is admitted. On the other hand, they have been several times reported by investigators, among others by Morgan, in *Drosophila,* and by the writer, in mice. In this connection it is interesting to note that the white doves referred to are not totally unpigmented being merely dilute, a fact easily observed in their eye color and found by Strong (1912) to hold true for their plumage.

2. *In Canaries.*—The factors involved are the allelomorphic P for dark eye color and p for pink-eye color. The quantitative relation between the two is somewhat similar to that described in doves though considerably less marked. The factorial change appears to be extremely rare and to be from the p to the P condition. The following results would be expected if the change occurred in the pink-eyed male.

| | Pink-eyed Male ppFFMM | × | Dark-eyed Female PpFFMm |
|---|---|---|---|
| Forming gametes: | pFM commonly<br>PFM exceptionally | | PFM<br>PFm |

Zygotes expected: (1) PpFFMM = dark-eyed males, (2) ppFFMm = pink-eyed females — commonly
(3) PPFFMM = dark-eyed males, (4) PpFFMm = dark-eyed females — exceptionally

Here as in doves the homozygous and heterozygous dark-eyed males would be distinguishable only after a carefully controlled breeding test. The dark-eyed females would be the only exceptional phenotypically distinct color class expected.

If the change occurred in the dark-eyed female instead of in the pink-eyed male we should have the following condition.

| | Pink-eyed Male ppFFMM | × | Dark-eyed Female PpFFMm | |
|---|---|---|---|---|
| Forming gametes | pFM | | | |
| | | | PFM | commonly |
| | | | pFm | |
| | | | PFM | exceptionally |
| | | | PFm | |

| | | | |
|---|---|---|---|
| Zygotes expected: | (1) PpFFMM = dark-eyed males | | commonly |
| | (2) ppFFMm = pink-eyed females | | |
| | (3) PpFFMM = dark-eyed males | | exceptionally |
| | (4) pPFFMm = dark-eyed females | | |

Here again *dark-eyed females* are the only unusual phenotypic color class produced.

In the reciprocal cross we should have the following:

| | Dark-eyed Male PPFFMM | × | Pink-eyed Female ppFFMm | |
|---|---|---|---|---|
| Forming gametes: | PFM | | | |
| | | | pFM | commonly |
| | | | pFm | |
| | | | PFM | exceptionally |
| | | | PFm | |

| | | |
|---|---|---|
| Zygotes expected: | (1) PpFFMM = colored males | commonly |
| | (2) PpFFMm = colored females | |
| | (3) PPFFMM = colored males | exceptionally |
| | (4) PPFFMm = colored females | |

As in doves the dark-eyed females of class (4) would be expected when crossed with ordinary white males, to produce *dark-eyed females,* an unusual color class, as well as dark-eyed males, a usual one.

*It will then be seen that the hypothesis of factorial change accounts for all the observed facts and unlike the hypothesis of partial sex-linkage or that of non-disjunction expects neither exceptional phenotypically distinct color classes as yet not obtained, nor any exceptional degree of sterility.*

On the hypothesis of factorial change it should be possible to obtain at rare intervals colored doves from white parents. None of those who reported exceptional colored females have reported this event. Nor have breeders of pink-eyed canaries recorded a dark-eyed bird from a pink-eyed × pink-eyed mating. In neither case, however, have a considerable number of young from such matings been reported by breeders *from animals of the same stock as that which gave the exceptional dark females.* It therefore remains quite probable that such a result could and would be ob-

tained as it was in Morgan's white-eyed flies and in my gray-bellied agouti mice.

Further, it would appear that another possibility, visionary though it may be, exists. If factorial change within a given locus is in any way influenced by other genes or combinations of genes within the cell either during gametogenesis or immediately after fertilization, we should expect that the w or p gene, as the case might be, would be subject to different intra-cellular environment when its allelomorph W or P was present, from that in which it would be placed in a homozygous ww or pp individual. Some of the differences which are bound to exist might well make for its relatively greater instability in the former as compared with the latter case.

*Although such a relationship is highly hypothetical, it is suggested that we should be continually on the alert for evidence of possible effects of intergenic and intra-cellular environment as one of the most probable causes of genetic change.*[3]

## CONCLUSION

It is believed that the extension of the hypothesis of partial sex-linkage and of non-disjunction, the effects of which have been clearly demonstrated in *Drosophila* should be made to include other forms only after confirmatory genetic and, wherever possible, cytological evidence have been obtained, and in the absence of any other hypothesis which equally fits experimental facts and is capable of experimental proof.

It is therefore suggested that the occurrence of occasional colored females in a cross between white male and colored female

[3] It should be recognized that sex-linked inheritance gives opportunity for the recognition of factorial changes should they occur, to a far greater extent than ordinary crosses—for example: In in a case *not* involving sex linkage we cross an individual homozygous or heterozygous for W with a w individual, the small w might change to its epistatic allelomorph W in rare cases without being recognized unless each of the supposedly Ww zygotes resulting from the cross were tested individually and sufficient young obtained to determine whether they are exceptional WW individuals. This has not been done on any very large scale with either birds or mammals under experimental conditions.

If, however, the change occurred in a cross involving sex linkage, it would be at once evident in at least one type of mating. This, as we have seen, is the cross of white wwFFMM male by WwFFMm colored female where any change in the w factor in the male would at once become evident by the production of colored females otherwise not expected.

doves, and of dark-eyed females in a cross between pink-eyed male and dark-eyed female canaries may be due to a rare factorial change from the factor w to its allelomorph W in doves and from the factor p to its allelomorph P in canaries. Such a change would account for the observed results, except no sterility nor additional unrecorded phenotypes and would be subject to experimental tests.

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# SHORTER ARTICLES AND DISCUSSION

## TRICHOMONAS AND BLACKHEAD IN TURKEYS

In reading the introduction to Dr. E. E. Tyzzer's contribution in the May issue of the *Journal of Medical Research* entitled "Developmental Stages of the Protozoon of 'Blackhead' in Turkeys," one is almost certain to be left with the impression that the conception of the agency of the common flagellate, *Trichomonas,* in producing pathological conditions characteristic of blackhead in turkeys, as described in several papers by the present writer, has no legs to go on, and would scarcely receive the consideration of sane protozoologists. Of course this is not the impression that Dr. Tyzzer meant to leave; so that it is fortunate that, in the experimental section of the paper referred to, he makes certain observations which are more favorable to the "flagellate hypothesis." Fearing, however, lest the hypothesis of tissue-invasion by *Trichomonas* might as yet be too frail to survive long under the criticism of two such men as Dr. Tyzzer and Dr. Theobald Smith (formerly chief proponent of the "Amebic theory"), the present writer, who first had the misfortune seriously to mention *Trichomonas* in connection with blackhead, wishes to point out a few instances in which Dr. Tyzzer's criticisms, real or implied, are due either to careless reading of the original papers, or to a too hurried examination of the plates, or to both.

In way of introduction it may be said that Dr. Smith's first exposition of the blackhead disease, together with his original description of the causative agent, *Ameba meleagridis,* appeared in 1895. It is true that, at that time, as Dr. Tyzzer states, the possibility of the relationship between *Ameba meleagridis* and the flagellates was suggested by Dr. Smith. And the suggestion was expressed in these words:

> There is probably no genetic relation between this hypothetical organism (flagellate) and the true parasite of the disease under consideration.

For twenty years the "Ameba hypothesis" stood; and it was not until this interpretation was called into question by Cole and the present writer that (as Dr. Tyzzer states), Dr. Smith explained "that the name 'Ameba' is employed tentatively, and

that it may be necessary to change this when the nature of the parasite is better understood.'' The present writer would probably have been more cautious in his original criticisms of Dr. Smith's conclusions if that statement had been made by Dr. Smith in 1895 instead of 1915. This will answer a criticism of Dr. Tyzzer's that is rather subtle and only implied.

Again, it is implied that something must be wrong with an investigation that purports to demonstrate that *Trichomonas* is the causative agent in an infection, when the investigator can not put his finger on the species concerned, or even risk the foundation of a new species. Who will come forward and give us a clear, definite and usable classification of the Trichomonads! And, speaking of new species in such a poorly known group, one can not help wondering if it would not have been just as well, in an earlier case, to leave the ''*meleagridis*'' off of *Ameba.* Can one doubt that many unhappy hours and profitless discussions have resulted from the necessity of piling up premature adjectives after an innocent Latin noun? Why embarrass the lexicographers until we are sure? And in the case under consideration the present writer wasn't sure.

Again Dr. Tyzzer states that it is obvious that the present writer ''does not consider the organism as primarily pathogenic in nature, but as a normal inhabitant of the alimentary tract of turkeys and fowls which may invade the tissue under conditions which lower the resistance of the host.'' This is quite true. Then Dr. Tyzzer continues,

> Apparently this author attaches no importance to the fact that the disease may be produced in healthy flocks by the introduction of infected birds.

This is also quite true. The writer does not know of a carefully controlled experiment in which it has ever been conclusively demonstrated that blackhead has been produced in healthy flocks by the introduction of infected birds. During one year of experimental work in the field the writer made it a point of removing the ceca and livers of poults which died of blackhead, chopping them up in a meat cutter, mixing lightly with middlings and feeding *en masse* to other poults as a partial substitute for beef-scraps. The mortality from blackhead in the fed group and in the control group was essentially the same. Most of the poults died after several weeks with gape-worm infection, and with no sign of pathologic changes in either ceca or liver. The

writer would have no apprehension in feeding to "healthy" poults any reasonable amount of pathologic material from blackhead cases, *provided it were done in such a way as not to upset the normal digestive equilibrium, and not to introduce pathogenic bacteria nor bacterial toxins.*

If Dr. Tyzzer had seen as much of blackhead in the field and on the farm as he has seen in the laboratory, he might more readily find reason in the writer's viewpoint. Until a few years ago, the writer held strongly the same views which Dr. Tyzzer now holds. But here, as in some other branches of science, "field work" and field experience has often wholesomely corrected misguided laboratory theory; at least the writer has found it so in his own case.

In another place Dr. Tyzzer states:

Contrary to Hadley's claim *Amœba meleagridis* should not be regarded as a cell parasite. . . . It does not occur within cells except after motility is lost, when it is soon phagocyted.

Regarding the matter of cell-invasion Dr. Tyzzer quotes from a passage from the author but stops prematurely. The passage should be read as a whole to obtain the writers' full meaning. The writer states that, in tissue-invasion, we see *Trichomonas* in a new rôle, and that here it may actively invade living cells. At this point Dr. Tyzzer's quotation stops, but in the original the text proceeds:

One may remark that the type of cell invaded is a highly specialized type [endothelial], and one that, by its nature, is more or less open to invasion.

The writer points out elsewhere that this invasion is not passive but active. But nowhere in any of his published papers (except in reference to the "goblet" cells) does the writer give any expression of the opinion that *Trichomonas* is a cellular parasite in the same sense that applies to the coccidia or other sporozoa. In this respect, Dr. Tyzzer accidentally misrepresents the writer's views. Such little mistakes as always likely to happen in the hurried reading of long and complicated papers.

A further criticism of Dr. Tyzzer's is too good to omit. The circumstances are as follows: The epithelium of the cecum of the turkey is thrown into folds. Sometimes they are deep and sometimes shallow. Within the folds, next to the cecal wall, are the crypts. The projecting folds, with their accompanying tissues,

the writer has referred to as the villi. The author points out that invasion of the submucosa is brought about by the passage of the flagellates through the epithelium of the fundus of the crypt, and that secondarily they invade (from behind) the villi, and finally escape into the cecal lumen after pushing off the epithelium of the villus tips. This phenomenon, which can be followed clearly in suitable sections, the writer has referred to as the stage of "reversed infection," and has pointed out that it constitutes a means whereby the parasites complete their parasitic cycle, rather than being buried and destroyed within the tissues as stated by Dr. Smith, who is of the opinion that the parasite of blackhead lacks this essential feature of perfect parasitism.

Here is Dr. Tyzzer's criticism of this exposition:

> The fallacy of such reasoning is quite apparent when the facts of the case are considered. There are no villi in the portion of the cecum commonly involved in blackhead.

The writer had carefully explained in the text the appearance of the invaded tissues; he had pictured it by hand-drawings, and more in detail by a series of photomicrographs. No one could fail to understand the definite histological structure to which the writer referred, whether it is properly termed a "villus," or something else. Dr. Tyzzer may call the histological structure what he pleases. The facts of the case with reference to *Trichomonas* remain the same.

But leaving aside the propriety of the term, villus, let us consider what Dr. Tyzzer means by the balance of his sentence ". . . in the portion of the cecum commonly involved in blackhead." In the examination of hundreds of cases of blackhead in turkeys and wild fowl the writer has found that blackhead lesions may be initiated anywhere in the cecal wall; there is no part of the cecum that is "commonly involved" except for this circumstance: the majority of the lesions are observed in the distal half of the cecum. Thus Dr. Tyzzer neglects clearly reported facts to grapple with a technical triviality in nomenclature; and at the same time, manifestly from lack of experience with many cases of the disease, misrepresents one of the essential facts relating to cecal infection.

In the next sentence Dr. Tyzzer attacks the statements of the writer regarding the avenue of infection of *Trichomonas*. Re-

ferring to the separation of the epithelium from the basement membrane, he states:

> In one case the separation of the epithelium is taken as evidence of invasion, and in the other it is taken as evidence of escape of the flagellates from the tissue.

Dr. Tyzzer quite mistakes the point involved. It is not the separation of the epithelium that is the important point (since this is often an artifact), but the orientation and grouping of the parasites in the vicinity of this epithelium. By looking at a church door we can scarcely tell whether the last congregation went in or out, but if we can find the congregation the question will probably be answered.

And in further criticism of this point (avenue of infection and of exit) Dr. Tyzzer has the misfortune to state,

> The organisms interpreted by Hadley as encysted forms of the flagellate being discharged from the tissue are evidently *Blastocystis* derived from the cecal content.

Did Dr. Tyzzer fail to examine the writer's photomicrographs (Bulletin 168, Figs. 30, 32 and 36) together with the complete description of these figures on a preceding page? Did he fail to read the description of this "reversed infection" on page 26? Are the writer's photographs so poor as to make possible a confusion between a flagellate trophozoite and "*Blastocystis*," or has Dr. Tyzzer an inadequate conception of what *Blastocystis* really looks like? And, in addition, may it not be a little inaccurate to affirm that "there is now quite general agreement that they (*Blastocystis*) represent a distinct type of organism . . ."? The matter is apparently still in controversy.

As to the statement of Dr. Tyzzer that the writer has failed to establish the identity of the parasite with any species of *Trichomonas*, or "to demonstrate any features characteristic of the genus,"—this must be left for others to judge. But the author can not forbear to reiterate that he has no reason to withdraw the evidence presented in previous papers. The strongest evidence of all comes from the relatively rare cases in which one can trace from the beginning the movements of the parasites in the tissues, and follow clearly the morphological changes that they undergo as the infection proceeds. It would seem that Dr. Tyzzer, in his examination of only "five infected turkeys," has never seen such cases. The present writer worked for many years before he found the ideal specimens. It is a

hard thing to realize, in such an investigation where one is attempting to ascertain the relation of two widely different entities, that a single average case, even though admirably sectioned and stained, may mean very little. Dozens of cases usually afford a more comprehensive view; and finally one comes to be able to piece together bits of information which make the story clear. It would be miraculous if the keenest pathologist could make clear the evidence from "five cases." Protozoan life histories are not read in a moment, and a study of a hundred cases for an hour means much more than one case for a hundred hours, —unless that one case is exceptional.

In concluding, it may be added that the writer hopes later to consider more in detail the valuable *constructive* aspect of Dr. Tyzzer's paper. It is freely admitted that the life history of *Trichomonas* in the tissues is not wholly clear, and it seems possible that some of the forms referred to by Dr. Tyzzer are new. This is especially true of some of the motile stages which, in the tissues, lose their flagella and, as Schaudinn says, "auch mit stumpflobosen Pseudopodien umherkreicht." It will probably be some years before the last word is said on the blackhead problem; and yet we are progressing. Under an efficient smoke screen Dr. Tyzzer has given the last blow to the "Amebic theory" and already—though grudgingly—has yielded some support the agency of the flagellates in cecal and liver infections. It may be confidently expected that in the course of time his researches will give more.

Dr. Tyzzer closes his critical introduction with the following words: "It may appear that the above discussion is unduly critical of the findings of other investigators. The confused state of the subject, however, appears to warrant drastic methods and the singling out of various misinterpretations and inconsistencies, for it is quite evident that the enthusiasm of certain investigators for their views has caused them to neglect important facts."

How we all wish to be such champions of the truth! But, in our war on "misinterpretations" and "inconsistencies" and on "neglect of important facts," would not our scientific world be a happier place, and all our work of greater merit, if criticism were tempered more with keen insight and less with the ardent spirit of academic chivalry? PHILIP HADLEY.

KINGSTON, R. I.,
October 9, 1919

## THE INTENSITY OF ASSORTIVE PAIRING IN CHROMODORIS[1]

THE pairing of the hermaphroditic nudibranch *Chromodoris zebra* is accomplished in such a manner that there occurs a considerable degree of assortive conjugation with respect to size. A report[2] presenting evidence in support of this conclusion was based upon the examination of *Chromodoris* population in Great Sound, Bermuda, at a season when a considerable percentage of

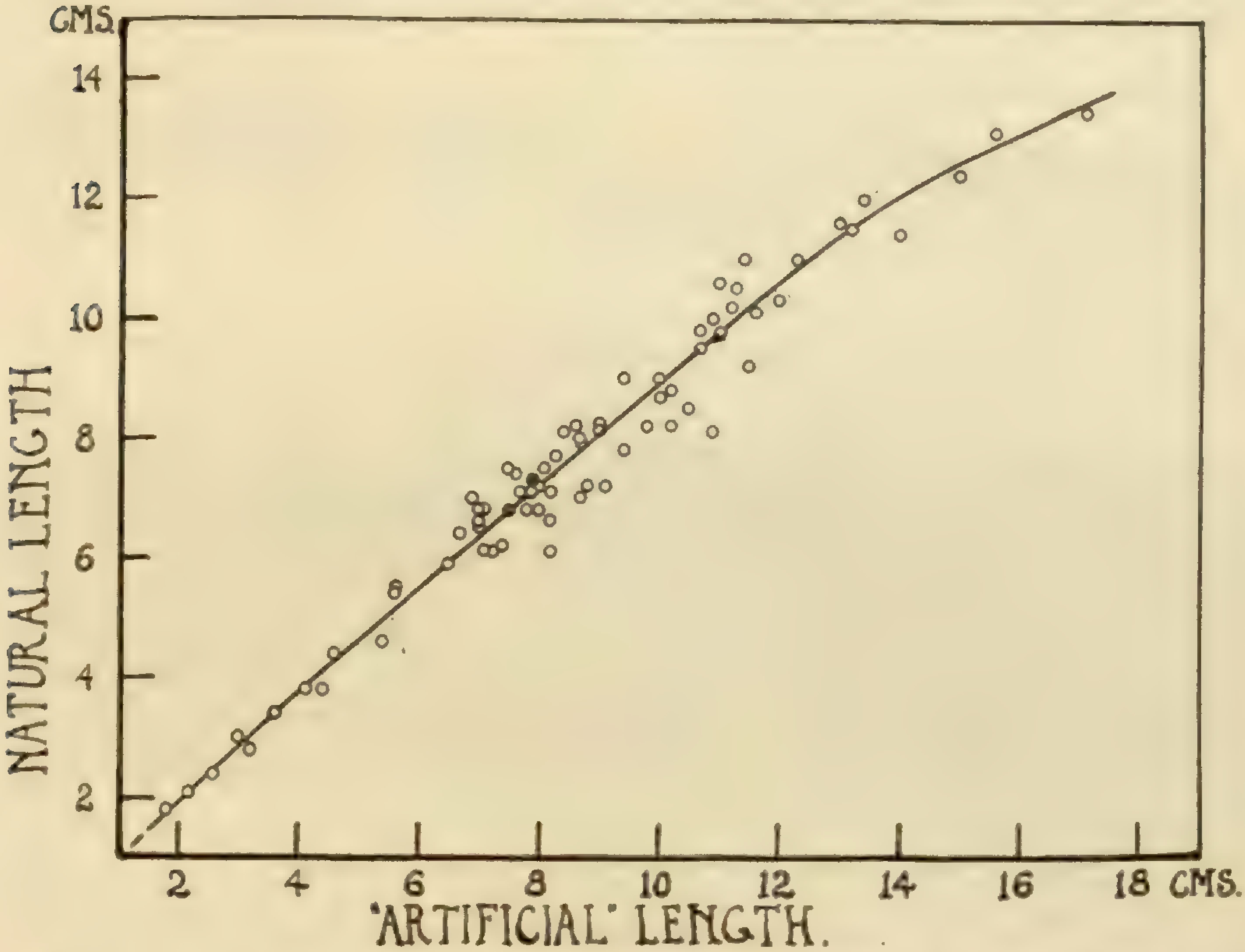


FIG. 1. Curve relating the total length of *Chromodoris* of various sizes to the "length" as obtained from an "artificial" method of measuring the length (see text).

the individuals exhibited injuries of the dorsal region of the mantle. These injuries, resulting in a distortion of the dorsal part of the body, made it necessary in estimating size to measure the total length of the animals—from anterior edge of the buccal veil to posterior termination of the foot. For practical purposes it was necessary at that time to employ a somewhat artificial

[1] Contributions from the Bermuda Biological Station for Research, No. 115.

[2] Crozier, W. J., "Assortive Mating in a Nudibranch, Chromodoris zebra Heilprin," *Jour. Exp. Zoöl.*, Vol. 27, pp. 247–292, 1918 (cf. *Proc. Nat. Acad. Sci.*, 1917, Vol. 3, pp. 519–522).

method in measuring this length. The animals were placed, dorsal surface downward, upon a glass plate freshly wetted with sea-water. It was recognized[2] that the soft body of these nudibranchs was by this procedure flattened out, and to some extent increased in length, and that the proportionate amount of distortion might be different for animals of different sizes. Opportunity was therefore subsequently taken to establish the relation

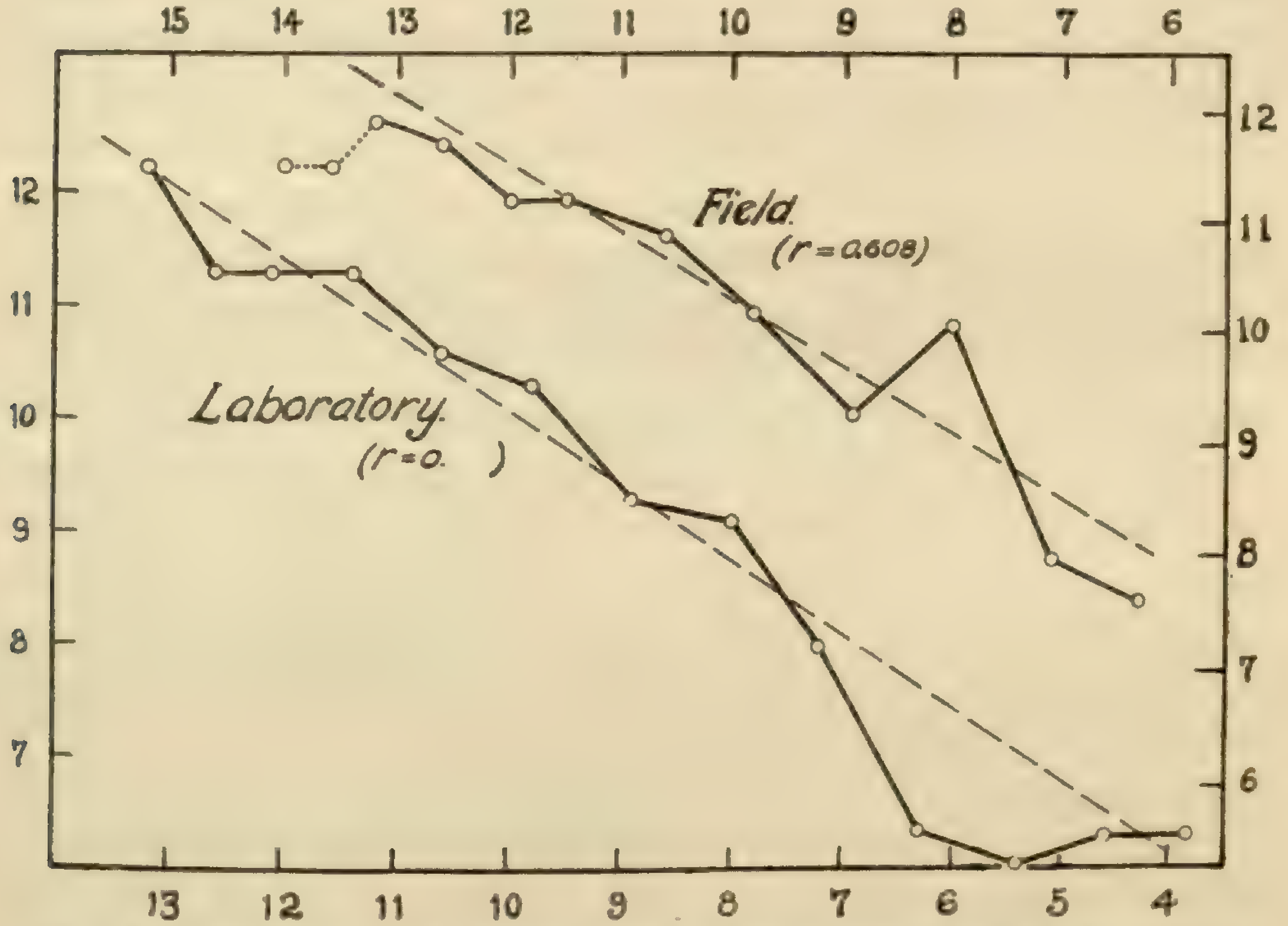


FIG. 2. Regression plots; upper, from 148 pairs copulating in nature; lower, from 119 pairs formed in aquaria; abscissas, length-classes in cms.; ordinates, the mean lengths of the mates of the nudibranchs in each of the corresponding length-classes; length measurements reduced by means of Fig. 1. The regression lines are those previously found (see 2), employing the length as "artificially" determined, and on the assumption that the regression is essentially linear.

between the "artificial" length as previously measured, and the total length of the nudibranch as normally creeping on a flat surface. The lengths of 74 individuals were determined in each of these ways. The result of these measurements is exhibited in Fig. 1.

It is apparent that with nudibranchs of the larger sizes the "normal" length is 1–2 cm. less than the length as artificially estimated; further, that, as was to be expected, the extent of the distortion introduced by the latter method is proportionately greater in larger specimens, the effect being negligible below 3 cm. Accepting the curve as a measure of the relation desired,

Fig. 2 contains regression plots for my data[2] on pairs found in nature and for laboratory matings in mass experiments, the length-classes having been redistributed according to their respective values in terms of the "normal" length. This procedure involves the assumption that the proportion of flattening in the "artificial" method is the same for animals of the same size-class at different seasons, which is probably not quite exact. The original records were obtained in April–May, 1917, whereas the data for Fig. 1 were secured in September, 1918. In the presence of so many possible sources of variation as these measurements permit, it is sufficient to "average" the determinations graphically, each original length-class, and the corresponding mean length of the mates of individuals in this class, being treated as units in reducing the old "length" figures to the more natural ones obtained through Fig. 1.

According to Fig. 2, the apparent intensity of homogamy in Chromodoris is but little affected, if anything perhaps slightly improved, by the reduction of the original figures to the natural scale.

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## THE ORIGIN OF THE INTOLERANCE OF INBREEDING IN MAIZE

The marked intolerance of inbreeding in maize has recently been discussed by Collins[1] and brought to the support of the hypothesis that this plant is of hybrid origin. But to those who look for the origin of maize in another direction, the problem is capable of a very different solution.

Briefly stated, Collins' argument is this: Most varieties of maize suffer from a few generations of self-pollination, but teosinte does not seem to be affected by this treatment. The maize plant as a whole is usually synacmic, with a tendency toward protandry, and self-pollination is in a large degree possible; such inflorescences of maize as have both stamens and pistils are distinctly protogynous. In teosinte the large number of inflorescences on a single plant makes self-pollination a common thing. If maize arose from teosinte, what was the origin of its intolerance of inbreeding? The assumption that maize is

[1] Collins, G. N., "Intolerance of Maize to Self-fertilization," *Jour. Washington Acad. Sci.*, 9: 309–312, 1919.

of hybrid origin takes care of this difficulty by attributing this genetic peculiarity to the unknown parent, which hybridized with teosinte, and in which cross-pollination was probably secured by protogyny.

Three fallacies render this argument inapplicable to the problem that it attempts to solve:

1. We are at once confronted with a question as to how maize, embarrassed by its well-known intolerance of inbreeding and by the extensive self-pollination with which Collins characterizes it, has persisted through the ages. The answer is that self-pollination in the plant is not so common as would be inferred from Collins' discussion.

It is true that a single isolated plant is largely self-pollenized, if pollenized at all, but data derived from the single-stalk culture often practised in experimental work can not be accepted as a criterion, for maize is normally grown in hills, and probably has been for a very long time. This method of cultivation is described by every early explorer and writer on Indian agriculture and seems to have been the rule from the garden beds of the Great Lakes region to the terraced mountain slopes of Peru. In many instances as many as eight or ten plants were grown in a single hill. This was the outgrowth of the Indian's limitations in the way of implements and domesticable animals, and the plant was well adapted to it. The method was adopted by civilized man and is extensively employed, with but few modifications, to the present day.[2]

If all the plants in a hill were synacmic and flowered at the same time and the air were motionless, the chances for self-pollination would vary inversely as the number of plants in a hill. Tendencies toward protandry, coupled with slight differences in the time of flowering of the individual plants of a hill, the prevalence of winds, and the proximity of other hills increase the chances for cross-pollination. Growing the plants in hills also discourages the production of suckers, thus reducing the number of inflorescences on a single plant, and consequently the chances for self-pollination.

This massing of plants together in hills, and of hills together in fields, is admittedly an artificial element of environment; but its possible evolutionary effect in the ages during which it has

[2] Cates, H. R., "Farm Practice in the Cultivation of Corn," U. S. Dept. Agr. Bull. 320, 1916, pp. 19–21.

prevailed can not be disregarded. What the condition was in wild maize no one knows, and there is little basis for speculation. But the plant has probably been in cultivation quite long enough to have had its character shaped by agricultural practise.

Directly in accord with this theoretical consideration are Waller's researches,[3] which indicate that when corn is grown in hills under ordinary field conditions, self-pollination occurs, on the average, in only a little more than five per cent. of the seeds. As Waller suggests, these figures may be modified by further work on the problem. There is no evidence, however, that the percentage of self-pollination will ultimately be found to be significantly larger than this. The genetic complexity of the average plant selected at random from any ordinary agricultural variety of maize is a standing evidence of the prevalence of cross-pollination.

2. The origin of protogyny in the androgynous inflorescences of maize need not be sought outside the Maydeæ. This is the regular condition in *Tripsacum*, at least in *Tripsacum dactyloides*, which is the only species that I have had opportunity to examine in flower, and it occasionally occurs in *Euchlæna*. Collins[4] and Kempton[5] disregard or question the existence of androgynous inflorescences in the latter genus, but the fact of their occasional occurrence remains. The lowest inflorescences of a teosinte plant are almost always wholly pistillate, and the highest wholly staminate. Perfect flowers have not been observed. but between the pistillate and staminate units androgynous inflorescences often occur. These are regularly protogynous. Androgynous inflorescences terminating the main culm are often produced in the greenhouse. The difference between greenhouse plants and those grown in the open in Mexico or southern Florida is fully appreciated. Androgynous terminal inflorescences are certainly of rare occurrence there, if they occur at all; but I am not sure but that they are of less frequent occurrence also in maize grown in tropical or sub-tropical countries. Monœcism in the Maydeæ is readily influenced by environment. The physiological conditions conducive to androgyny in the tassels of maize, and to a relative increase in the number of pis-

[3] Waller, A. E., "A Method of Determining the Percentage of Self-pollination in Maize," *Jour. Amer. Soc. Agron.*, 9, 35–37, 1917.

[4] *Loc. cit.* Also Collins, G. N., "The Origin of Maize," *Jour. Washington Acad. Sci.*, 2: 520–530, 1912.

[5] Kempton, J. H., "The Ancestry of Maize," *Jour. Washington Acad. Sci.*, 9: 3–11, 1919.

tillate flowers in *Coix* and *Sclerachne*, also produce androgyny in the inflorescence of teosinte. This fact may be of some significance. Maize was doubtless originally a tropical plant. How much of its erratic floral behavior when grown in temperate latitudes is due to real, fundamental differences between it and teosinte, and how much to environment?

It seems, then, that as to androgyny or as to protogyny of the individual inflorescence, there is no fundamental difference between maize and the other American representatives of the Maydeæ. When this fact is coupled with a reduction in the number of inflorescences, as in maize, it becomes unnecessary to assume the introduction of the intolerance of self-pollination from another group.

3. Collins leaves in this paper, as well as in an earlier one,[6] the impression that the alternative to his hybrid origin hypothesis is the theory that maize originated as a mutant from teosinte. The latter idea is quite as chimerical as the former. We can not reasonably hope to find the ancestor of maize in any modern plant; phylogenetic histories seldom work out in this way. The logical procedure is to look for other plants which may have descended, coordinately with maize, from a common ancestor. In *Tripsacum* and *Euchlæna* we find two genera that fill the requirements in all known details.[7]

The intolerance of inbreeding in maize is probably the plant's natural evolutionary response to its environment. The maize plant is unique among the grasses in bearing but one pistillate and one staminate inflorescence, or at most only a few inflorescences of each type, in having these widely separated, and in having been grown in hills for untold centuries. These conditions all tend toward extensive cross-pollination, and the data at hand indicate that cross-pollination is the rule. More or less adjustment to these structural characters and this mode of living would be expected; and the decline in vigor, resulting from inbreeding, may be interpreted as the natural consequence of an abnormal and unfavorable condition.

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[6] Collins, G. N., "Maize: Its Origin and Relationships," *Jour. Washington Acad. Sci.*, 8: 42–43, 1918.

[7] Weatherwax, Paul, "The Evolution of Maize," *Bull. Torrey Club*, 45: 309–342, 1918.

# NOTES AND LITERATURE

*Orthogenetic Evolution in Pigeons.* Posthumous works of C. O. Whitman, edited by OSCAR RIDDLE. Publication No. 257, Carnegie Inst., Wash. 3 quarto vols. with numerous colored plates and figures. 1919.

In the opening sentence of volume 1 of this notable publication, Whitman says "Progress in science is better indicated by the viewpoints we attain than by massive accumulation of facts." The viewpoint which Whitman himself attained and beyond which he saw no reason for advancing is that of "orthogenesis." His persistent industry also accumulated a mass of facts rarely surpassed in amount concerning variation in a single group of related organisms, the pigeons of the world.

The enormous task of setting these facts in order so as to illustrate his viewpoint, he was unable to accomplish. Death overtook him while he was still busy accumulating facts. But he was fortunate in having a loving pupil willing to devote his life, if necessary, to rescuing from oblivion the work and words of his beloved master. Few literary or scientific executors have shown such self-forgetting devotion or have seen it crowned with such success. Whatever we, living in a period of rapid advance in biology, think at present concerning the value of Whitman's viewpoint, there can be no doubt that Riddle has preserved it permanently, so that no one will be at a loss to know what Whitman's ideas were about the factors of evolution, or on what data they rested.

Whitman took as the point of departure in his pigeon studies, the plumage pattern of the wild rock-pigeon, *Columba livia,* made familiar to everyone by Darwin's use of it in his writings on evolution. Darwin supposed that the wild rock-pigeon of a slate blue color and with two black wingbars was the original form from which all varieties of domestic pigeons had originated through variation and selection. He showed that domestic varieties when intercrossed frequently revert to this wild type and he uses the manifold variation of domestic pigeons as a capital illustration of evolution through descent with modification. Whitman, in the true spirit of science which seeks to "try all things and hold fast [only] that which is good," made inde-

pendent studies of wild rock-pigeons obtained from the "Caves of Cromarty, Scotland." He found that not all the wild pigeons of this locality are of the simple two-wing-bar type, but that part of them show a different pattern known as "chequered." In these also the two black wing-bars can be observed, but they are rendered less conspicuous by the occurrence of other black spots scattered over other parts of the wing, giving the whole a chequered appearance. The wing-bars are due to the occurrence of a black spot on the tip or below the tip of each of two rows of feathers that lie across the wing when it is folded. In chequered birds other rows of feathers bear spots but the spots fall less regularly and obviously into rows, so that the pattern is more like that of a chequer-board. Further in young birds Whitman observed that practically all the wing feathers may bear spots, although in the later plumage some of the spots may disappear. He concluded that *this* condition was the primitive one, rather than the two-wing-bar type which Darwin regarded as primitive. This conclusion seems well founded since the chequered type is thus seen to be less specialized in form and earlier in ontogeny. So far Whitman's work supported Darwin's general evolutionary ideas, merely improving a detail in one of his illustrations, and showing that there still exists among wild pigeons a pattern yet more primitive than the one which Darwin had taken as the point of evolutionary departure. But Whitman now extended his investigations to other species of pigeons and finally to those of the entire world to see if he could work out more fully the evolutionary history of plumage patterns in the group. As a result of these studies he reached conclusions which did not enter into Darwin's scheme of evolution. The most important of these is known by the name of "orthogenesis." This is the idea that evolution through natural selection does not result simply from the selection of chance variations, that variations do not occur in *all* directions but only in particular directions in *straight lines* from the point of departure, hence the name *orthogenesis.* Whitman's study of the plumage patterns of pigeons is probably the most extensive, as it is the most recent, of the studies of a group of animals made in the light of this principle, but to the general body of biologists free from bias for any particular theory it will scarcely be more convincing than its predecessors. It is possible to arrange any group of related organisms in a graded series and to assume that they have been evolved by orderly development, from one end of the series (either end) to the other;

but this is no proof that such has actually been the historic method by which the series has arisen. It may actually have started in the middle and worked both ways, or in several directions. Only a study of contemporaneous genetic variation can show what the method of evolution is. Color variation in mammals is not unlike that of birds. We might arrange the color varieties of any species of mammal or group of mammals in a linear series and assume logically enough that evolution had progressed from the darkest to the lightest form in orderly manner, or *vice versa,* yet the study of contemporaneous variation shows that this is not the case. A wild species, like the gray rabbit or the brown rat, undergoes sporadically genetic variations ("mutations") some of which are lighter, some darker than the parental form. They have no relation to each other as to the order, time, or place of their appearance, so far as we can discover. Breeding evidence shows that they are genetically independent one of another.

As an alternative to the hypothesis of orthogenesis in variation, the mutation theory of DeVries received much critical consideration in Whitman's writings. The lateness of publication of much of this is to be regretted. Discussions which might have been helpful a few years ago are now quite superfluous and out of date in the light of critical experimental evidence since produced.

Mutation has practically ceased to be considered as a hypothetical method of the immediate and direct origin of species. Even as regards the origin of characters, mutation is no longer supposed to be a simple process. Whitman maintains with entire correctness that " unit-characters" often have small beginnings and may later be gradually increased by systematic selection. Frizzling of the feathers in pigeons and fowls is an example cited by him. He says, p. 151:

> Minute frills may occur in one or two feathers only, and they may occur in any number, or in all of the feathers. . . . The full character is reached, not by a jump, but by a process of modification, carried farther and farther, from the initial starting point. . . . It is well known that characters often disappear by degrees, not all at once. In crossing species we rarely find the hybrid with *pure* characters. A character may be halved, quartered, etc., to any fractional part of the original.

In passages such as these Whitman clearly shows that the muta-

tion theory as held at that time was untenable when applied either to the origin of species or to the origin of characters. What has since happened is that the mutation theory has been frankly abandoned as applied to such origins and is now limited to the origin of factors or genes. It is recognized that characters may change progressively and permanently (just as Whitman believed they did) under the guidance of selection. The agency of such change is now supposed to be modifying or multiple factors, so numerous as singly almost to baffle detection and so frequently coming and going that gradual modification of characters in a desired direction is not difficult. This is the residuum of truth which underlay the mutation theory as Whitman knew it and attacked it. In this marvellously modified form, he would probably not have attacked the theory at all.

Volume 2 deals chiefly with inheritance, sex, and color in hybrids of wild species of pigeons. An enormous amount of experimental data is here recorded, and scattered notes, briefs for lectures, etc., have been brought together by the editor, dealing with such general topics as heredity, Mendelism, sex determination and the like. As regards the hybrids, only $F_1$ individuals were produced, for Whitman says, p. 3,

> In the case of the wild species of pigeons, of which there are nearly 500, crosses are very often infertile, and fertile hybrids are so rare that Darwin could not find a single well-ascertained instance of hybrids between two true species of pigeons being fertile *inter se*, or even when crossed with one of their pure parents. The records since Darwin's time have not furnished the instance he vainly sought for.

Now every one to-day realizes that the $F_2$ or second hybrid generation is all important for understanding or interpreting heredity. Whitman accordingly, notwithstanding the boasted superiority as genetic material of the pure species with which he worked, since he was unable to produce in any case a second generation of hybrid birds, had no adequate basis for discussing heredity in his hybrids, and no adequate basis for criticizing Mendelism which is revealed only in the $F_2$ generation. One characteristic of the large number of sterile $F_1$ hybrid birds which Whitman produced is noteworthy. Their characters were in nearly all cases blends or intermediates between those of the respective parents. So long as doubt remained as to what the significance of blending is, whether it is essentially different in nature from Mendelian inheritance, Whitman thought rightly

that he had grounds for questioning the universality of Mendelian inheritance. But strong evidence has now been produced that blending inheritance is the regular outcome of crosses involving multiple factorial differences. $F_2$ in such cases shows increased variability with occasional segregation of the extreme parental types, and in $F_3$ and $F_4$ such segregation becomes more common. Had Whitman been able to raise $F_2$ and $F_3$ generations, he would undoubtedly have been convinced, contrary to his expectations, as some of us have been, that blending inheritance finds adequate explanation in multiple factor Mendelian inheritance. It is true that Whitman's records of hybrid birds reveal sex-linked inheritance, but these records did not suffice for its discovery, which fell only to those experimenters who worked with the despised "domestic breeds." The most valuable part of the work recorded in this volume is probably the basis which it afforded for experiments on quantitative factors entering into the development and expression of sex, if not its actual determination. This work is due largely to the pupil and editor, Riddle, though he generously brings the name of the master to the front in dealing with the subject. These results have been dealt with more fully in other publications by Riddle and need not here be reviewed.

Volume 3 deals with very different subject matter from that contained in Volumes 1 and 2, viz., the behavior of pigeons. Here is subject matter for the trained animal psychologist and Dr. Riddle felt constrained to call in a competent psychologist to edit this portion of Whitman's writings. Professor H. A. Carr has rendered this important service in a highly acceptable manner. That a single biologist should be able to do distinguished work in two fields so distinct as genetics and animal behavior shows the breadth of Whitman's capacities and interests. The reviewer is unable to deal critically with the contents of Volume 3, but hazards the suggestion that it contains material of very great interest and of permanent value not only to the psychologist but also to the naturalist, the one who is interested in animals as animals rather than as examples and products of one evolutionary process or another.

It is much to be regretted that Professor Whitman was unable himself fully to develop and round out the field of work here so ably outlined and in part explored.

W. E. Castle

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# CHIASMATYPE AND CROSSING OVER

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Two short papers by Janssens, published in the *Comptes Rendus* of the *Société de Biologie* for April and May, 1919, outline an interpretation of the maturation-phenomena in Orthoptera in agreement with his earlier chiasmatype-theory ('09) based on the corresponding phenomena observed in urodeles. It is a matter of so much importance that all phases of this question be fully discussed that we venture to report and examine the conclusions announced in these new communications. For this purpose we have found it convenient to divide the discussion into two parts, one dealing with the matter more from the standpoint of strictly cytological observation, the other more from that of the possibilities suggested by genetic analysis. In order to avoid repetition we have numbered the figures consecutively, but each author is responsible for the part under his name.

## I

## A Cytological View of the Chiasmatype Theory

E. B. WILSON

Professor Janssens's results are as yet illustrated only by diagrams, which leave us in doubt concerning some very important details; nevertheless, a cytologist may be permitted to indicate at this time how the conclusions are related to those of other cytologists who have ex-

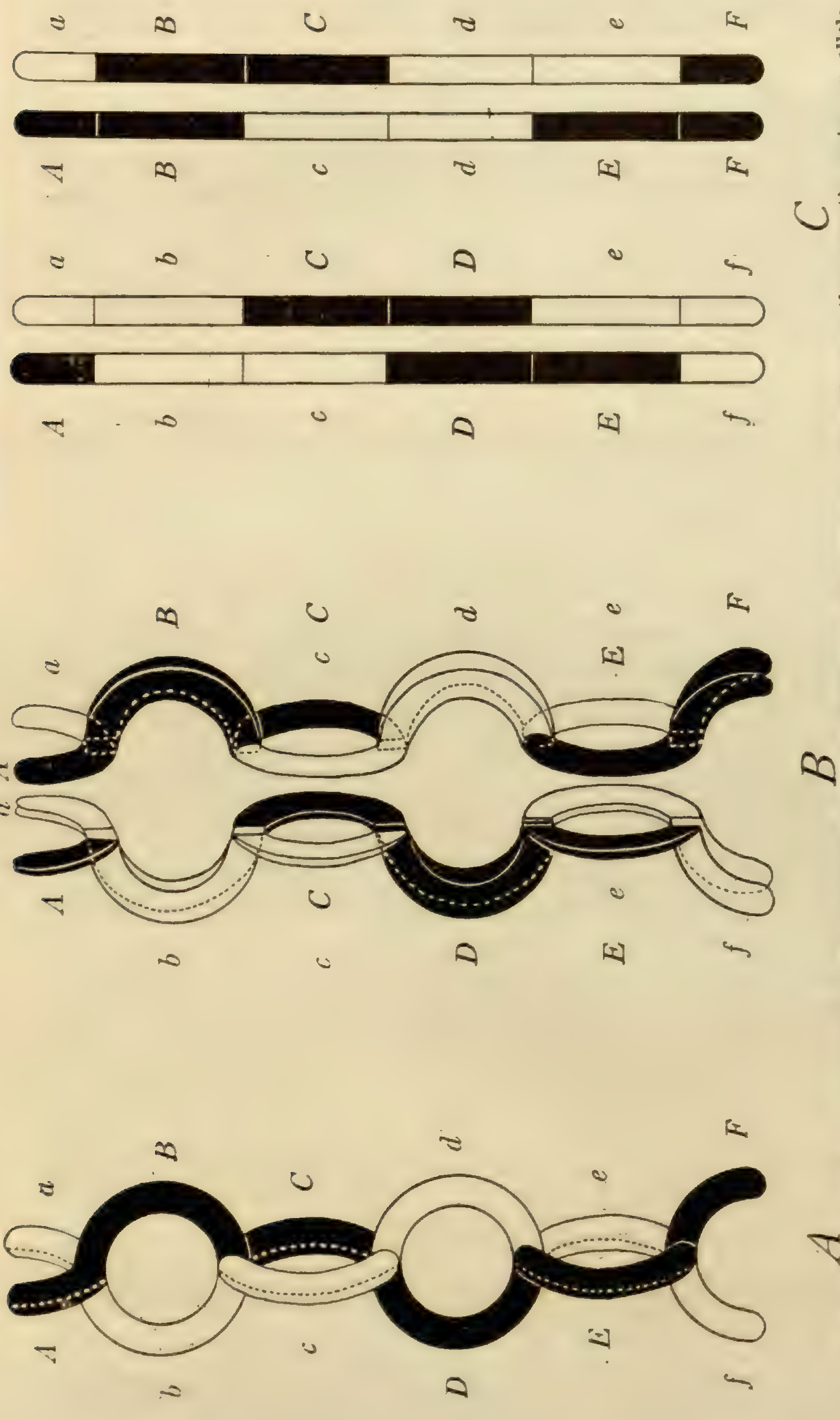


FIG. 1. Janssens's interpretation of the compound rings in Orthoptera (Janssens). In each case the synaptic mates or allelomorphs are in black and white respectively. Corresponding or homologous regions of these mates are designated as $A$ and $a$, $B$ and $b$, etc. $A$, the ring-series in perspective; $B$, its first division (the second division is at right angles to this, nearly in the plane of the paper); $C$, the four resulting classes of chromatids.

amined the phenomena in Orthoptera and other insects. Incidentally I may remark that Janssens cites from one of my own papers ('12) in support of his general theory and also copies from another ('13) a series of general diagrams by which the theory was illustrated. As he

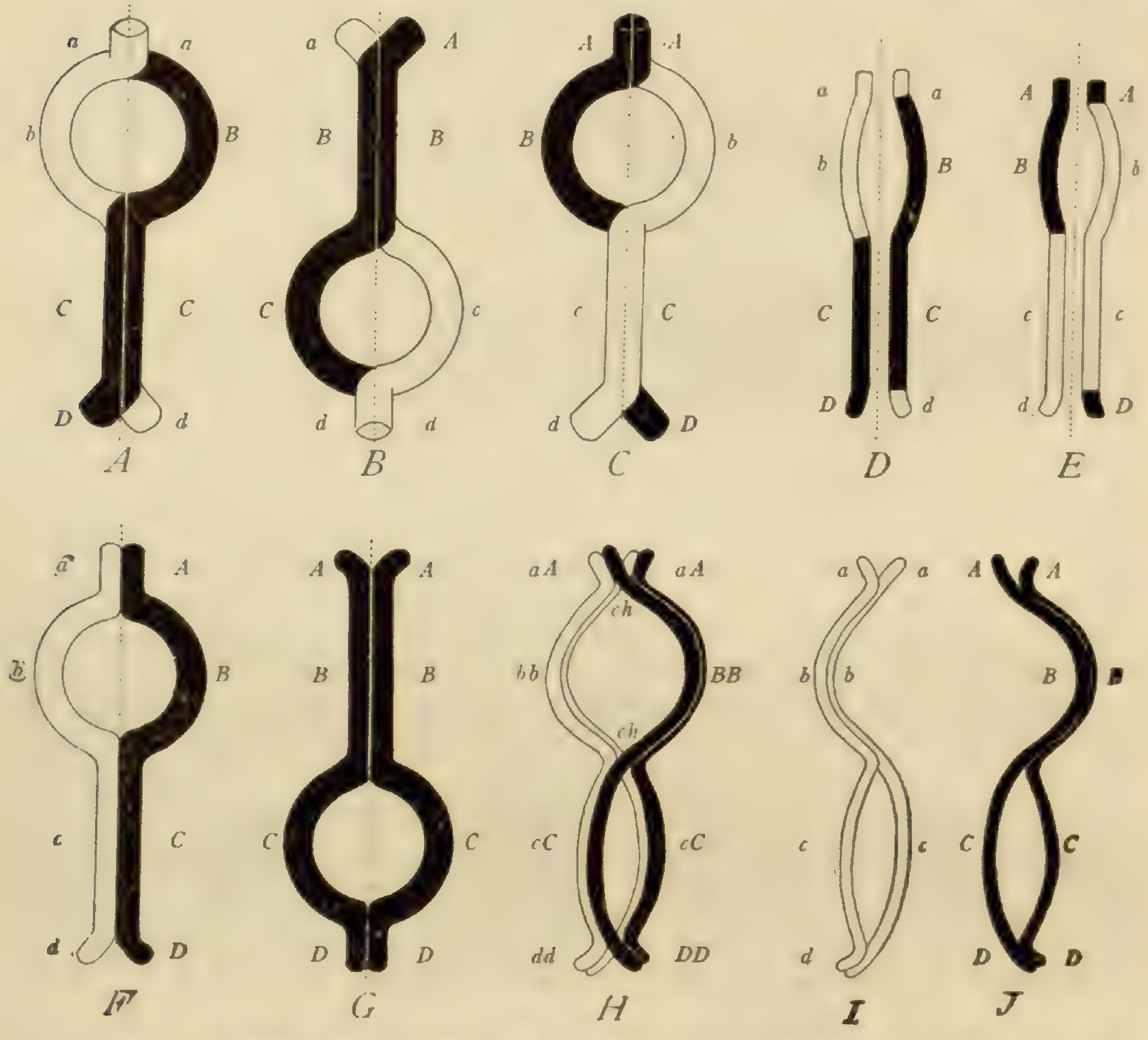

FIG. 2. History of the double rings (*A–E*, after Janssens). In each case the synaptic mates are black and white, respectively, corresponding regions marked *Aa*, *Bb*, etc., as in Fig. 1. *A–E* show Janssens's interpretation of their history. *A*, double ring from one side; *B*, the same rotated 90° to the left; *C*, the same after rotation through 180°; the dotted line shows plane of first division; *D*, *E*, the four resulting classes of chromatids; *F–H*, corresponding figures showing the composition of these rings as heretofore described; *I* and *J*, the two resulting classes of chromatids, with no cross-overs.

points out, these diagrams were too much simplified to give an adequate representation of his views; but a critical account of cytological *minutiæ* was obviously inexpedient in a presentation intended only to make clear to a general audience the nature of Janssens's fundamental assumption. I am glad however to see from these latest

papers that he does not consider the diagrams to have misrepresented the gist of the matter.

So far as can now be judged, Janssens's latest studies add nothing to his paper of 1909 that is new in principle. They are in the main an elaboration of his earlier conclusions concerning the double-ring and double-cross types of tetrads, which were illustrated in his former work by diagrams XI, XVI, XIX and XX. These two forms of tetrads are closely related, and each of them shows in the prophases of meiosis a two-strand chiasma —*i. e.,* two threads which seem to pass over from one synaptic mate to the other, crossing each other midway between them, as in Fig. 5, I or 5, III—such as formed the main basis of the original chiasmatype theory. It may be needless to describe these tetrads, which are perfectly familiar to cytologists, but for the sake of clearness I will briefly review their composition as now generally understood.

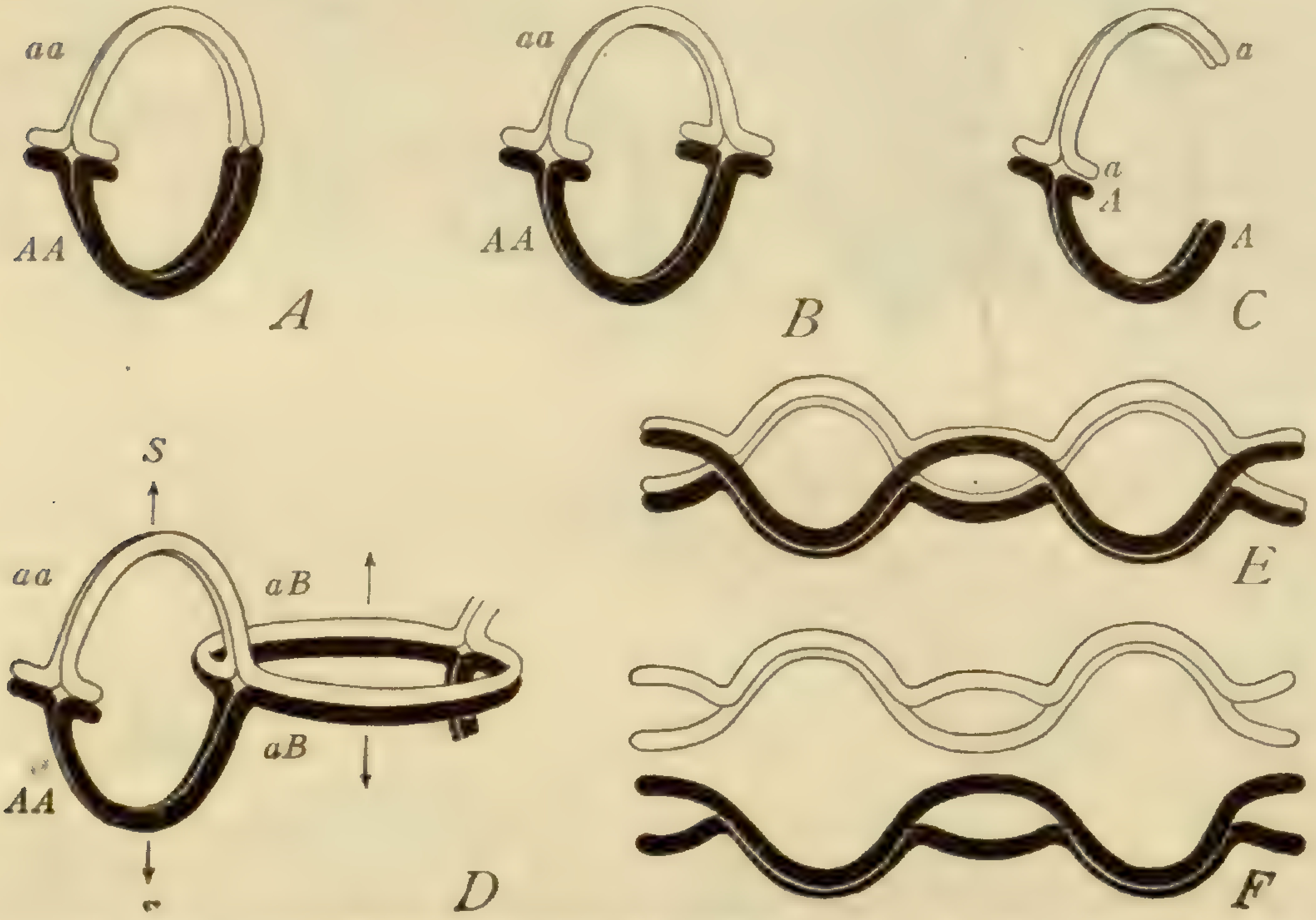


FIG. 3. Diagram to show relation between the single rings, double crosses, double ring and multiple ring types of tetrads. In each case the synaptic mates are black and white respectively. *A*, single ring with one pair of lateral arms; *B*, same with two pairs of arms; *C*, double cross type with curved arms; *D*, double ring, showing spindle-attachments (*S*, *S*); *E*, multiple ring type (viewed in somewhat different perspective); *F*, mode of division of such a tetrad.

Ring-tetrads may be single, or may consist of two or more rings joined together in such a manner as to be successively at right angles to one another, as is schematically shown in Janssens's diagram, here reproduced in Figs. 1 and 2 *A–C*. Single rings of the type here in question (Fig. 3 *A, B*) were I think first clearly described and figured in my laboratory by Paulmier ('98) in Hemiptera, though he did not correctly make out their mode of origin. Similar rings were subsequently studied in many other animals, *e. g.*, in Orthoptera by McClung, Sutton, Granata and others, in urodeles by Janssens, and in annelids by the Schreiners, Foot and Strobell and others. More recently they have been carefully examined by a number of observers, in particular by McClung ('14), Robertson ('16), and Wenrich ('16, '17). The single ring-tetrad (Fig. 3 *A, B*) consists of a more or less open ring, split lengthwise into two closely apposed halves and cut crosswise at opposite points by two sutures which divide the ring into two semicircular half-rings. The latter are now regarded by practically all observers (Janssens included) as the synaptic mates, joined by their ends but elsewhere widely separated so as to lie on opposite sides of the ring-opening, and each longitudinally split. The longitudinal cleft lies therefore in the plane of the future equation-division, the cross-sutures in that of the reduction-division. At one of the cross-sutures, less often at both, the longitudinal halves of both synaptic mates are commonly drawn out at right angles to the ring (in the manner made clear by Fig. 3 *A, B*) thus forming two lateral arms, each longitudinally double, so that this part of the ring, as seen in face view, offers the figure of a double cross. If the ring be supposed to break in two at the opposite suture and the half-rings to straighten out completely it would become a simple double cross-figure with two short arms and two long (Fig. 3 *C* or 5 III). If, on the other hand, the lateral arms of a closed ring be supposed to elongate still more and to bend away from the original ring until they meet,

they would give rise to a second ring continuous with the first but at right angles to it, as shown in perspective by Fig. 3 *D*; and by repetition of this process would be formed a series of three or more interlocking rings, each at right angles to its successor (Fig. 3 *E, F*).[1] How many such successive rings may be formed is not known. Double rings seem to be the most frequent; but in *Chorthippus* (*Stenobothorus*) both Robertson and Wenrich describe and figure triple rings including at least one case in which the lateral arms are long enough to form a fourth ring, though their ends are in fact free. Janssens's diagram (Fig. 1) represents four complete rings with lateral arms at both ends of the series; and it is quite possible, as McClung has suggested, that some of the forms that have been described as twisted or strepsinema stages may really be early conditions of such multiple rings.

Janssens has found that in the heterotypic division the double or multiple ring-tetrads lie on the spindle with their longer axis transverse to that of the spindle, and establish a lateral (atelomitic or non-terminal) attachment; and since successive rings are always at right angles to one another they lie alternately either in the equatorial plane of the spindle or in a plane at right angles to it, *i. e.*, tangential to the spindle. In the ensuing division the series is cut straight through in the equatorial plane (as shown in Fig. 1), rings which lie in this plane being split lengthwise while those lying tangentially are cut crosswise. This curious result is perfectly in agreement with Robertson's observations on *Chorthippus* ('16, Figs. 179–182) and those of Wenrich on *Trimerotropis* ('17, Plate 3, Figs. 17, 18), and we may probably accept it without hesitation, at least for some tetrads of this type.

Thus far all observers are in agreement concerning the external structure and mode of division of the compound

[1] This account does not correctly describe the mode in which these compound rings actually arise, but it is a convenient way of making clear their structure.

rings. As soon as we look further we encounter what seems at first sight to be a hopeless contradiction between the conclusions of Janssens and those of others.

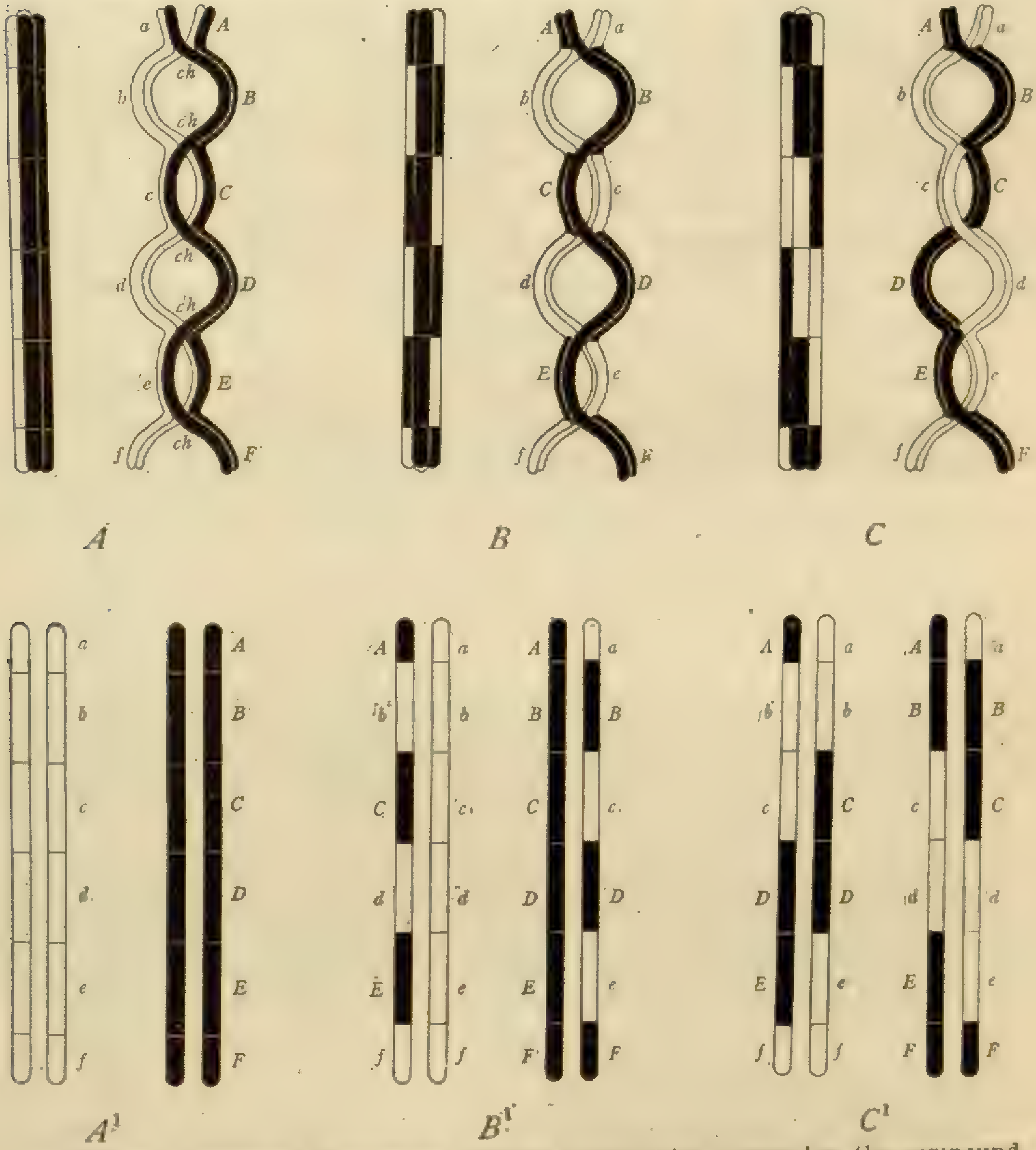

FIG. 4. Diagrams illustrating various possibilities concerning the compound rings, following the outlines of Janssens's figures, but showing also the relations of the chromatids. At the left in each of the upper figures is the longitudinal tetrad-rod from which the ring-series arises, showing results of assumed early cross-overs in $B^1$ or $C^1$. *A*, the compound ring as conceived by McClung, Robertson, etc., with the four resulting chromatids at $A^1$ (no cross-overs). *B*, a compound ring, such as might follow a two-strand cross-over at each node, giving the results shown in $B^1$. *C*, a compound ring giving the results shown in Janssens's diagram (Fig. 1), resulting from a two-strand cross-over between two pairs of threads, in regular alternation at successive nodes. The result ($C^1$) is four classes of chromatids, as shown in $C^1$.

Janssens, holding fast to the general interpretation outlined in his earlier development of the chiasmatype-theory, considers the compound rings to have resulted

from a process of torsion of the synaptic mates about each other, followed by a partial fusion between them at certain points where threads from opposite sides of the spiral have come together, crossing each other to form a "chiasma" at each such point. By a subsequent readjustment of position the regions between these points of partial fusion have opened out to form rings disposed at right angles to one another, and connected at the points where the chiasmas have been formed. The general nature of this rather complicated conception may better be grasped by a study of Fig. 4*C* than from a description. Janssens assumes, further, that *at some period* in their history the rings are cut through at these points of fusion in such a manner as to effect an exchange of corresponding regions between the synaptic mates. The effect, as conceived by Janssens, is shown in Fig. 1 (copied from Janssens), and more in detail in my interpretative Fig. 4 *C, C′*.

Janssens's general interpretation (as will at once appear from his diagrams here reproduced as Figs. 1 and 2 *A–E*) includes two more specific assumptions on which the whole matter turns. These assumptions are: (1) that all the rings are essentially alike, the synaptic mates, or corresponding regions of them (black and white in the figure) lying in every case on opposite sides of the ring-opening, the longitudinal cleft in each thus representing the future equation-division; from this it follows (2), that rings which lie in the equatorial plane of the spindle (horizontally) are divided equationally, while the alternate rings that lie tangential to the spindle are cut crosswise, and hence reductionally, *by the same division.* Both these assumptions differ wholly from the results of previous investigators and hence call for critical examination.

The genesis and later history of the compound (especially the double) rings has been most fully studied in the Orthoptera, having first been considered by McClung and Granata, and more recently investigated with greater

precision especially by Robertson ('16) and by Wenrich ('16, '17). None of these observers, it is true, has traced the history of the rings in every detail; but their results, so far as they go, are entirely in harmony with the better known history of the single rings and double crosses,

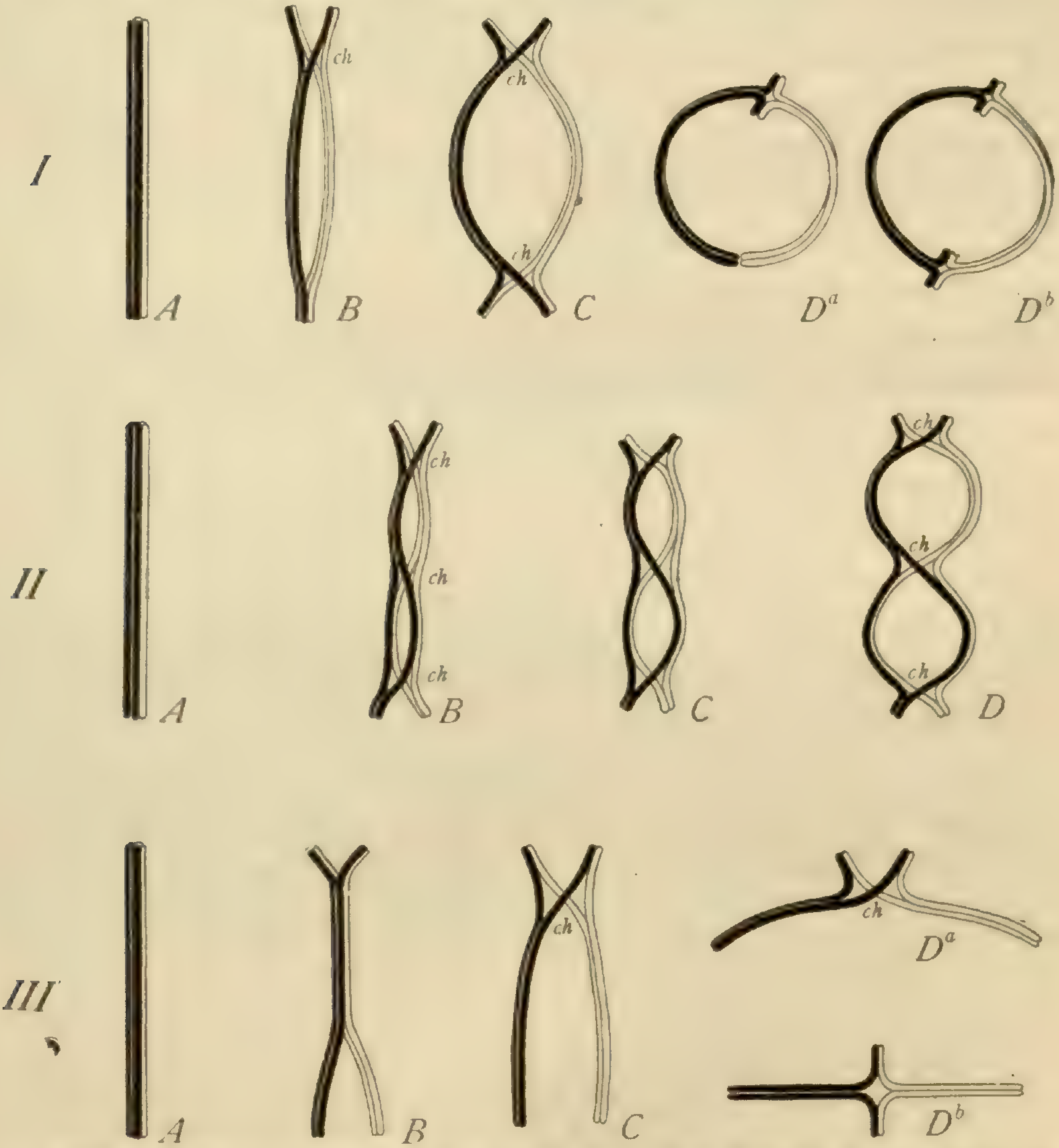


FIG. 5. Diagram (perspective views, from clay models) of the origin of single rings, double rings and double crosses from a longitudinally quadripartite rod. I, single rings; *B* leading to *Da*, and *C* to *Db*. II, double ring-formation. III, double cross-formation; III, *C* derivable from I, *B* by separation of the lower ends of the synaptic mates. In each case *ch* marks an apparent crossing-point or "chiasma."

both of which offer essentially the same problem as the compound rings. These various forms of tetrads arise from a diplotene thread that is at first longitudinally double and sooner or later longitudinally quadripartite

owing to the appearance of a second cleft at right angles to the first. The evidence is nearly or quite conclusive that one of these clefts coincides with the original plane of synapsis or side-by-side apposition of the synaptic mates (also the plane of the future reduction-division) while the other is the equation-plane along which each synaptic mate is longitudinally split.[2] In any case it is generally agreed that single rings arise by the separation and opening out of these threads along *one* of the clefts (generally believed to be the synaptic, as in Fig. 5 I), their ends remaining united, while the second cleft remains as the longitudinal cleft of the ring and represents the plane of the equation-division. The lateral arms of these rings arise, as shown in the figures (5 I, *B, C*) by separation and divergence of the free ends for a certain distance along the second (equational) cleft, thus finally giving the appearance of a double cross at this part of the ring (5 I, *D*).

Double rings, coupled together (Fig. 5 II) arise when the rods separate along different planes in two adjoining regions, the opening of one ring representing the expanded synaptic cleft (appositional or reductional) that of the other the equation-cleft. Such rings are of course at right angles to each other; and as the diagram shows (Fig. 5 II, *B, D*) when these tetrads are viewed obliquely they seem to show at certain points crossed threads or chiasmas (*ch.*) in which two threads cross over from opposite sides.[3] It is of the first importance, however, to bear in mind the fact that *such figures are shown in fore-shortened view.* They are an attempt to represent in two dimensions a figure which actually is in three dimensions. Such tetrads can not adequately be visualized until modeled in clay or by means of wires, so as to be seen in three dimensions. When the models are obliquely viewed they seem indeed to show at each node

[2] This interpretation disregards the possibility (which I think is a probability) that recombination-phenomena or orderly exchanges of material between the synaptic mates may already have occurred in the quadripartite rod; but for the moment we may leave this out of account.

[3] This is clearly shown in McClung's photograph, Fig. 122 ('14).

two threads that are connected by a chiasma and two that are not thus connected; but if the model be rotated through an angle of 90° the appearance is reversed, the "chiasma" now appearing between the two threads that previously seemed unconnected, and *vice versa.*

The same appearance, due to the same cause, is given in early stages of the lateral arm-formation in the single rings (5 I, *B, C*), and is shown with even greater clearness in early stages of the double crosses. The latter arise by separation of the free ends of the four threads from each end towards the middle point, but along different planes (Fig. 5 III, *B–D*), *i. e.,* from one end along the equation-cleft, from the other along the reduction-cleft—a process that is continued until all four threads come to lie in a single plane in the form of a double cross. Here, too, a "chiasma" (*ch*) is very clearly seen; but as in the foregoing cases it is an optical illusion; the models in three dimensions show at once that a straight split through the tetrad involves no transverse break in the chiasma, and that its two strands merely draw apart as the division proceeds. In themselves these figures give no reason whatever to assume that such a break (crossing-over) has taken place at an earlier period or that the synaptic mates have been twisted about each other, as Janssens assumes.

Such an origin of the double or multiple rings seems at first sight wholly inconsistent with Janssens's interpretation; for if it be correctly determined the relation of the synaptic mates to the ring-formation is wholly different in successive rings, as is shown in Figs. 3 *D, E,* 2–*H,* and 4–*A.* Specifically, in case of any two successive rings one always shows the synaptic mates, lying on opposite sides of the ring-opening, and each longitudinally split, while in the adjoining ring half of each synaptic mate surrounds the entire ring-opening, lying in close contact with the corresponding half of its mate. Only in the first case, accordingly, does the longitudinal cleft of the ring correspond to the equation-division. In the sec-

ond case this cleft coincides with the apposition-plane of the synaptic mates (*i. e.,* that of the future reduction-division) while the equation-cleft has opened out to form the ring-opening; and so on in regular alternation. It follows, lastly, that if we disregard for the moment the possibility of an earlier recombination-process, a division that cuts straight through the tetrad, as described alike by Wenrich, Robertson and Janssens, does not in fact divide certain rings equationally and others reductionally in regular alternation but divides the whole series in the same way, either equationally or reductionally as the case may be (Figs. 2 *H, J,* 3 *E, F,* 4 *A*).

In order to make clear the contrast between this conclusion and that of Janssens I have in Fig. 4 *A* followed his outlines but have indicated the course of the four threads (chromatids) in accordance with the account just given. In Fig. 4 *C,* on the other hand, the chromatids are shaded black and white in such a manner as to fit with Janssens's account. A similar comparison is shown for the double-ring tetrads in Figs 2 *F, G,* which follow Janssens's outlines (2 *A, B*) as nearly as possible but are differently shaded; while 2 *H–J* shows the double ring and its mode of division in slightly oblique view, so as to show the "chiasma." In these various figures it is at once evident that although a two-strand chiasma or crossing (*ch*) appears at the junction of every two rings, a straight longitudinal division of such a tetrad (separating black from white) involves on crossing-over, and divides every ring reductionally; *i .e.,* in such a manner as to disjoin the synaptic mates. Here again it is also evident that the multiple ring need involve no twisting of the synaptic mates about one another. It is true that rings of this type, whether single or double, are not infrequently twisted in their earlier stages, and sometimes in their later—a fact long known and easily verifiable; it is shown unmistakably, for instance, in some of my own slides of *Phrynotettix* (from material given me by McClung several years ago). No evidence has yet been pro-

duced, however, to show that such torsion leads to double ring-formation by a process of chiasmatypy. On the contrary, the evidence thus far indicates that the torsion is undone as the prophases advance; and it is a significant fact that in these same twisted rings the free ends of the chromatids (forming the lateral arms) show the typical relation as described above, giving the appearance of a chiasma at each end (as in Fig. 3 *E, F,* or 5 II).[4] Such "chiasmas" (like those seen at the junction of two rings) are not for a moment to be confused with the appearance of crossed threads given in side views of actually twisted rings.

Such is the contradiction—at first sight it seems irreconcilable—between Janssens's conclusions and those of other investigators of these tetrads. These latter results, in particular those of Robertson and of Wenrich, are supported by very detailed and precise studies; and my own observations, particularly on the double crosses, are altogether in favor of their conclusions. Until Janssens's evidence is before us in greater detail it remains to be seen whether the contradiction really is as great as it now appears. In the meantime we may briefly consider certain possibilities which may help to define the issue more clearly.

The conflict of results has not, I think, grown out of the fact that Janssens has worked with a different type of compound ring, though this is possible, nor can we assume that he has not reckoned with the results of other observers. I incline to think that the contradiction may be in the main one of theoretical interpretation rather than of known fact; for in theory all the observed facts may quite logically be interpreted as the result of a chiasmatype that has been completed at a stage prior to the ring-formation. Specifically we might assume that a cross-over has earlier been completed at each node in the series, causing an exchange of two longitudinal halves in

[4] See for instance Granata ('10, Fig. 29), Robertson ('16, Figs. 150*b*, 175), Wenrich ('17, Pl. I, Figs. 8, 9), and Mohr ('16, Fig. 131). The same is clearly seen in my slides.

alternate rings—a process which would produce a condition identical so far as appearances go, in both structure and mode of origin, with the compound ring as described by Granata, McClung, Robertson or Wenrich, but one which has a quite different morphological significance. Such an assumption seems to me to be logically implied by Janssens's own account, though I am not sure that such is actually his meaning.

I have tried to illustrate this by the series of diagrams shown in Fig. 4. *A* represents the compound ring in accordance with the results of McClung and his followers, the synaptic mates being in black and white, respectively. If, however, we assume this condition to have been preceded by a two-strand cross-over or chiasmatypy at each node, the composition of the tetrad becomes that shown in *B* or *C*. Either of these figures realizes the two specific assumptions of Janssens earlier emphasized, namely: (1) that the longitudinal cleft in every ring represents an equation-division (*i. e.,* separates corresponding halves of one synaptic mate in this particular part of the tetrad), and (2) that a straight split through the ring-series (such as is shown in Fig. 1 *B*) will now divide half the rings equationally and the alternate rings reductionally. Both show recombinations in the same regions (*Aa, Cc, Ee*) and in the same relative numbers in the cross-over threads; but they are differently grouped, owing to the fact that in one case (*B*) a cross-over has taken place between the same pair of threads at every node, while in *C* this occurs only at every other node, the cross-overs taking place in regular alternation between two different pairs of threads. As a comparison of Figs. 2 and 4 *C* will show, it is this latter form that corresponds with Janssens's interpretation.

Janssens does not make it clear in his preliminary papers whether he assumes the chiasmas to be cut through during the actual division of the tetrad, though I think is what one would naturally infer from his general account and from his figures, especially of the double

rings (here reproduced as Figs. 2 *A–E*) and of the double crosses (here Figs. 6 *H–C*). On the other hand a study of my Fig. 4 will show that the results of the division, as stated by Janssens himself (my Fig. 4 $C^1$) can only be brought about by a split which passes straight through the equational cleft of the horizontal rings and leaves the chiasma untouched. I infer, therefore, that Janssens does in fact consider the chiasmatypy to have taken place

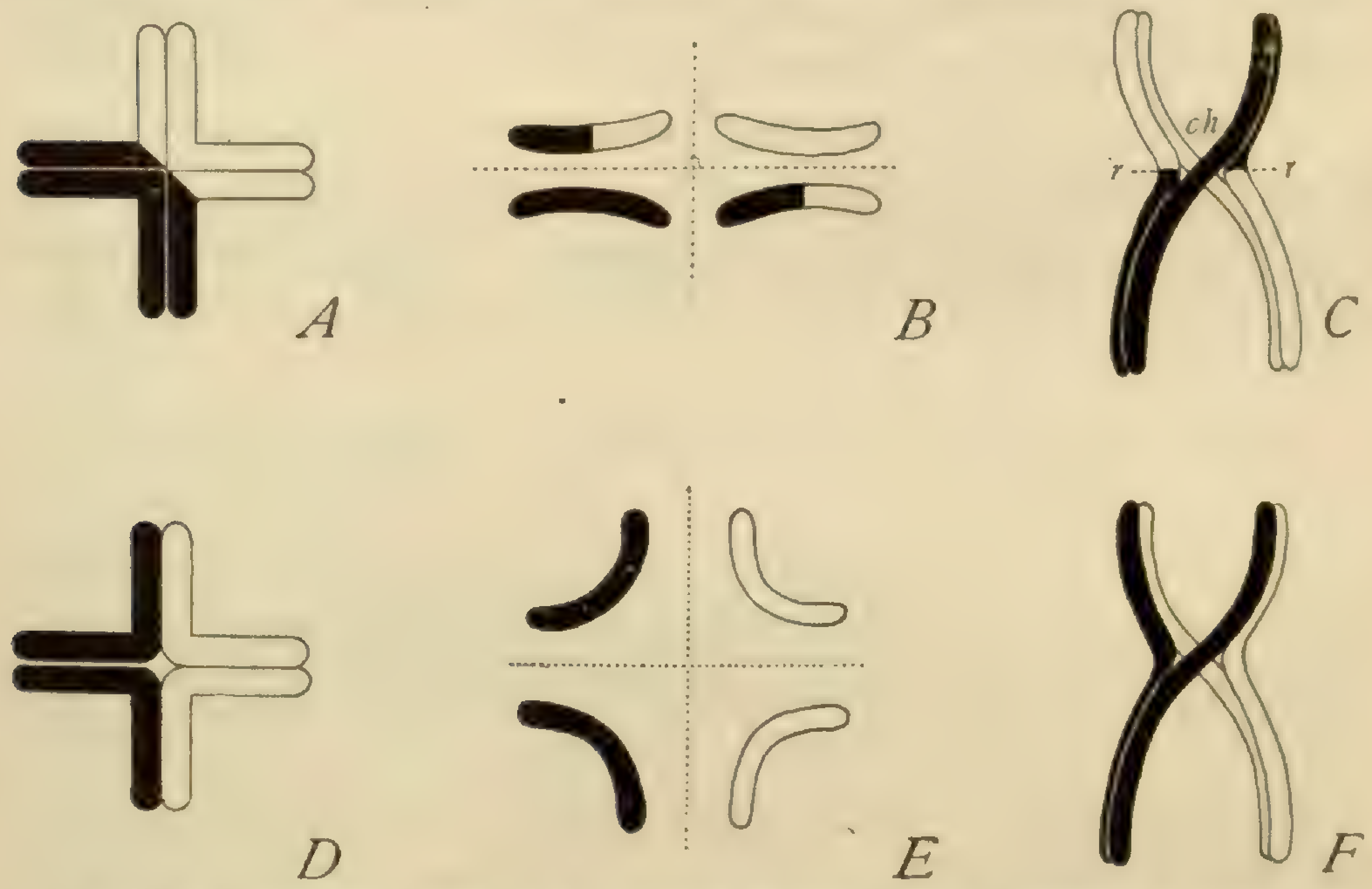


FIG. 6. Interpretations of the double crosses. *A, B,* Janssens's interpretation (from his figures), showing four classes of chromatids, two with a single cross-over each. The result is here the same as shown in Fig. *C* (after an earlier figure of Janssens). *D, E,* the prevailing interpretation of the double cross, with no cross-overs; *F,* early stage of the double cross. (*Cf.* Fig. 5 III, *C.*)

at a stage prior to the opening out and division of the rings; and this would be in agreement with his earlier conclusions, as applied to the tetrads of urodeles, here illustrated by Fig. 6 *C* (after Janssens).[5] At any rate, so far as I can see, it is only by such an interpretation that Janssen's results can be reconciled with those of other observers. More specifically, the assumption must be, I

[5] See Janssens, '09, p. 14 (Diagram, XXII). "We believe that in this case the threads which cross each other are those furthest apart, that is to say, which occupy those parts of the chromosomes that undergo no intermixture. The threads which remain unconnected by a chiasma, on the contrary, are those which have undergone a secondary union at the points where the chromosomes have interpenetrated each other and fused."

think, that the chiasmatypy has taken place during a strepsinema stage prior to the straight, longitudinally divided threads from which the rings arise (Figs. 4 *B, C*, at the left). If now, for the sake of argument, we accept these assumptions, how does it come to pass that the subsequent opening out of the rings exactly fits with the recombination-phenomena that have previously occurred in the tetrad? Morgan has already supplied an answer to this in the ingenious suggestion that *the mode of separation of the threads may be determined by their nature* —*i. e.*, that the paternal and maternal threads (or portions of threads) may always be the first to separate, however they may lie in the tetrad.[6] This is an important addition which makes the whole series of assumptions logically complete.

All this constitutes a somewhat complicated train of reasoning; nevertheless, if it be granted, it provides formally an escape from the seeming contradiction and leaves the chiasmatype-theory intact. The point, however, that I wish to emphasize is that we have now passed over into a realm of hypothesis and logical construction, based it is true on a vast assemblage of data of the highest importance, but derived from genetic experiment rather than from cytological observation. No observer, so far as I know, has yet seen a process of true crossing-over (recombination) by means of torsion, chiasma-formation, fusion, and secondary splitting apart. That such a process takes place at all remains thus far an inference based on the presence of a continuous two-strand chiasma in later stages of meiosis and on certain resulting appearances in the late prophase- and metaphase-tetrads. But as shown above, precisely the same appearance of a two-strand chiasma is given by a process in which no torsion need be involved. Both Wenrich and Robertson have urged this fact against Janssens's interpretation; and I am fully in agreement with them so far as the later stages of meiosis are concerned. It may nevertheless be pointed out that both these observers have figured stages

[6] '19, pp. 101–104.

which at least suggest a process of torsion or strepsinema-formation in the early diplotene prior to, or very early during, the definitive opening out of the prophase-figures—*e. g.*, in Wenrich ('16), Fig. 75, or ('17), Fig. 23, and in Robertson ('16), Figs. 149, *a* and *b*. The case seems, therefore, by no means closed; and we may await the publication of Janssens's new results in greater detail, in the hope that more definite evidence may now be produced concerning the critical point at issue.

My own doubts on this matter first grew out of observations on the origin of the double crosses, which, as above indicated, involve a similar question concerning the chiasmatype. Janssens's earlier interpretation of the double cross, which I believe he was the first to offer, was in principle the same as that briefly indicated above and schematically shown in Figs. 5, III and 6 *D–E*. Later this interpretation became the prevalent one but was abandoned by Janssens himself ('09, '19) in favor of one which assumes a process of chiasmatype to be involved in the cross-formation. This interpretation starts with a comparison of the double cross to the region at which two rings join; and this is obviously correct under any theory (cf. Figs. 3 *D* and 6 *C*, *F*). Janssens, however, assumes the relation between the synaptic mates to be essentially as shown in the diagram here reproduced as Fig. 6 *A–B*, the two synaptic mates being bent at right angles, and united by their apices to form a cross which then splits straight through all four arms, thus giving two cross-over chromatids out of four. I seriously considered this interpretation in my own studies on the double crosses of Hemiptera, but finally became convinced ('12 and subsequently) that it does not correspond with the facts. More recently Robertson, Wenrich and Mohr have demonstrated the same conclusion in a very circumstantial and convincing manner in case of the double crosses of Orthoptera, tracing their origin step by step from the original diplotene in the manner indicated in Fig. 5, III. According to all these observa-

tions there is nothing in the history of these crosses, as thus far made known, to suggest an earlier process of torsion, chiasma-formation, and recombination. They indicate rather that the cleavage of such a tetrad straight through its two clefts involves simply one reduction-division and one equation-division (Fig. 6 *D–E*).

Robertson has pointed out that many of those appearances in the prophase- and metaphase-tetrads on which Janssens's theory was originally based are susceptible of a much simpler explanation than is offered by the chiasmatype-theory, namely, that they are a result of "misfortune in the prophase," due to secondary displacements of torsions at this time. Experiments with clay models have convinced me that this point is well taken, in respect to some at least of these appearances. It should also be clear from the foregoing discussion that conditions resulting from the persistence of the so-called two-strand chiasma in the metaphase-figures are readily explicable without the assumption of an earlier process of chiasmatypy.

In this brief review and critique of the cytological aspects of the question, I have not intended to take up an attitude of opposition towards the chaismatype-theory considered as an explanatory principle in genetics. On the contrary, I am not able to escape the conviction that somewhere in the course of meiosis some such process must take place as is postulated by Janssens and by Morgan and his co-workers, though I must admit that this opinion rests less on cytological evidence than on genetic. I have wished only to discuss the possibilities of the existing cytological situation and to offer a counsel of caution in respect to the chiasmatype-theory in so far as it is based on conditions seen in the later stages of meiosis. This means no lack of appreciation for Janssens's brilliant and fruitful work, which has opened up so remarkable a new field of inquiry. But a theory of such fundamental importance calls for critical treatment, and on its

purely cytological side too much has sometimes been taken for granted by writers on genetics. It is, I think, highly probable that the cytological mechanism of crossing-over must be sought in some process of torsion and recombination in the earlier stages of meiosis—perhaps during the synaptic phase of slightly later—and that this process may leave no visible trace in the resulting spireme-threads. To accept this, of course, would mean that such conventionalized diagrams as those here offered (Figs. 3, 5, etc.) should be so modified as to indicate exchanges which have earlier taken place between the synaptic mates. It must be said, on the other hand, that the actual evidence of torsion during the process of parasynapsis is still very inadequate and receives no support from some of the most careful recent work. One can not avoid a suspicion that some internal process of torsion (or of rotation, as conjectured by Correns) may take place in the early pachytene before the duality of the diplotene becomes externally visible. Conjecture concerning all this will however be less fruitful than further cytological analysis. The truth is that for the time being genetic development of the chromosome-theory has far outrun the cytological. We are in no position to predict when the plodding progress of cytology may be able to close the gap; nevertheless we have every reason to hope that the physical mechanism of the recombination-phenomena may in the end prove to be accessible to decisive cytological demonstration.

## The Spiral Looping of the Chromosomes and the Theory of Crossing Over

T. H. MORGAN

In his two recent papers Janssens calls attention to certain details relating to the application of the findings of cytology to the interchanges between homologous sets of linked genes. The first paper is a restatement of the situation as it is generally understood to-day, and calls

for no special comment. It ends with the significant statement

> At our next meeting I shall point out that the theory of the chiasmatype allows for an interpretation somewhat different from the view of simple splitting of the threads in a single plane that passes through the axis of the entwined threads.

Concerning the point here raised by Janssens I should like to add that the "simple interpretation" was given mainly to escape the somewhat complicated scheme involved in Janssens's theory. In this way it was hoped to avoid a too detailed account of the process that calls for pictures not readily understood except by cytologists familiar with the changes that take place when the twisted threads shorten and move apart. Unfortunately the very simplicity of the statement led one critic to infer that the interpretation must be wrong because at the nodal points the plane of the split appeared as though it cut obliquely through each chromosome itself. To avoid this I represented in later diagrams the chromosomes as made up of beads, and in this way tried to show that at the node each bead and its allelomorph are not divided, but go each to a pole. Even this diagram may prove too simple; for, if at the time of twisting each thread is also split lengthwise into two strands it is possible that only two of the strands fuse at each node. In Janssens's scheme to be described below this secondary doubling of each thread is seen to be an important factor in the situation.

Janssens states that the matter is not simple:

> The loops (Fig. 1 *B*) and the half loops (Fig. 1 *C*) that produce the chiasma lead to profound modifications in the twisted threads. These modifications are already indicated in the prophases, but they only become evident in proportion as the dyads ripen and prepare to place themselves on the spindle. We can not describe this here, but let us state nevertheless that the chiasma segments are placed in planes perpendicular to the segments adjacent to them as indicated in diagram II, Fig. 1. Once this fact is clearly seen it is not essential to add much to Morgan's phrase, since it expresses what really takes place. It need only be said: (1) In both maturation divisions a cleavage takes place

only in the equatorial plane of the figure. The first of these cleavages is here indicated by a dotted line in Fig. 1 *A*. (2) Moreover this plane produces a longitudinal cleavage of the chromosome and hence is equatorial in each of the alternating chromosome segments (rings) that lie exactly at the equator of the spindle of the figure (like that which occurs at a gonial mitosis), Fig. 1 *A* and *B*. (3) Finally, since the two spindles of the two maturation divisions that follow rapidly are perpendicular to each other, one may further add that each dyad will be split during the maturation (maiotiques) division by two planes at right angles to each other. At the first division, the equatorial division plane is perpendicular to the axis of the heterotypic spindle and during the second division the plane is in space, parallel to the original axis of the same spindle.

Janssens suggests the following considerations that have an application to Mendel's laws.

Neighboring segments pass easily into the same chromosomes when the direction of the twist is constant. When a segment is long it may be considered as carrying a longitudinal series of qualities, in conformity with the ideas held by Morgan. On the other hand the qualities supposed to be carried by the chromosomal segments are distributed amongst the germ-cells as though they were carried by chromosomes really independent confirming the law of disjunction of the characters in the gametes (Mendel).[7]

Let us return to a further consideration of the diagrams that have been published to represent the methods of crossing-over. In Janssens's scheme, Fig. 1 *A*, four complete rings are represented and the division plane appears to cut through each node, although the important details of how this is done are not shown in the figure, but may perhaps be inferred from Fig. 1 *C*, where the four vertical strands show what is supposed to have taken place. Crossing-over is represented as having occurred at four nodes. In *Drosophila* the genetic evi-

[7] In this sentence Janssens seems to imply that his chiasma theory explains "free assortment" between genes in homologous pairs of chromosomes, but obviously if the genes are in linear order, great numbers of them will go over together in the segments between the nodes, or on each side of a node. Hence the phenomena of linkage that places a very great restriction on Mendel's second law of assortment. It is this feature that we have always regarded as of the utmost significance in our theory of crossing over. It is obviously implied in Janssen's chiasma theory also, and I can not but believe that Janssens must intend to apply his theory in the same way in which we have applied our theory.

dence shows that as much crossing over as this does not usually occur. In our diagrams (Heredity and Sex, 1913), therefore, we represent only one or two real interchanges between the members of a pair of chromosomes because the genetic evidence shows, as stated, that, in the great majority of cases, this is what takes place.

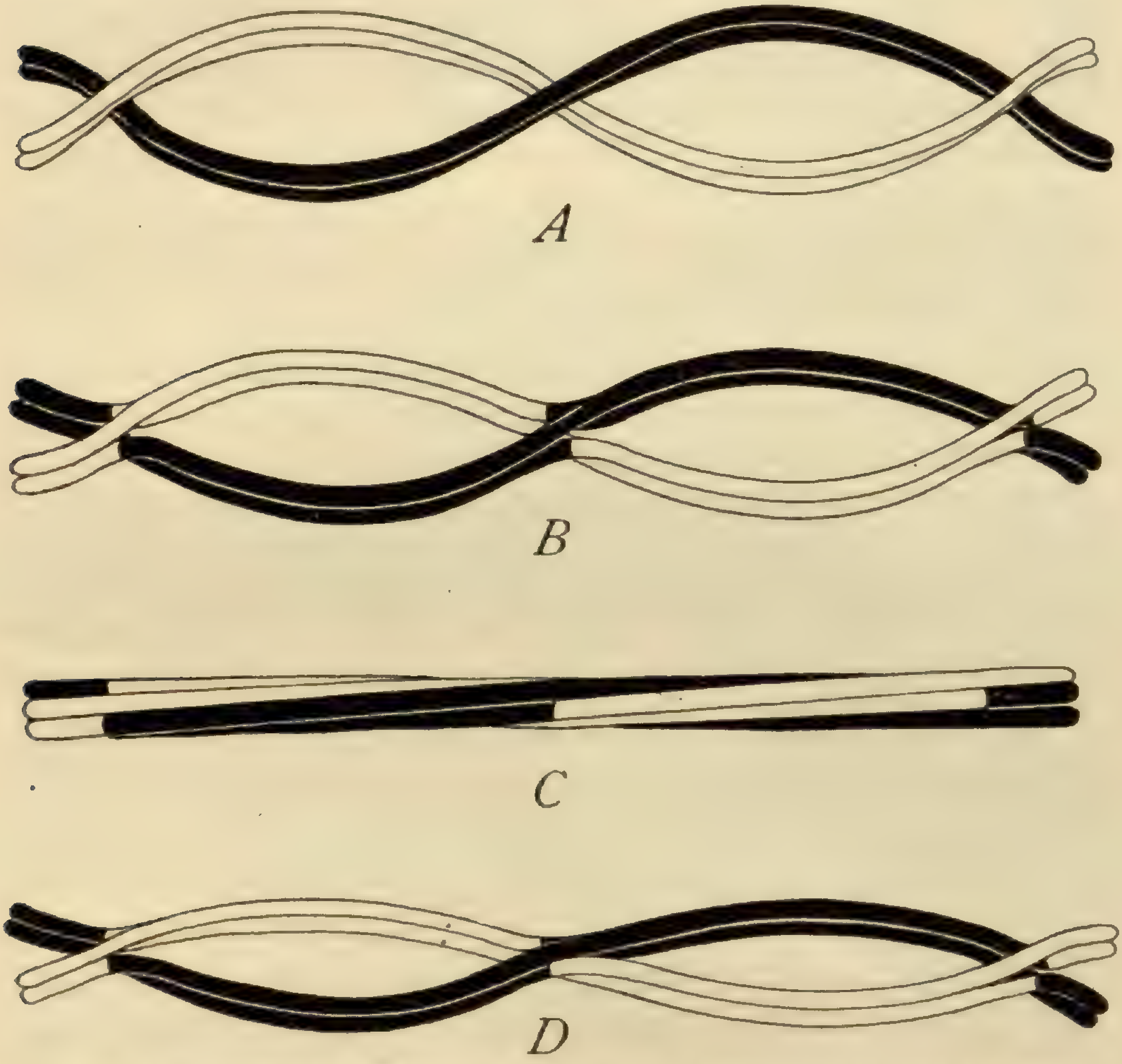


FIG. 7. Diagram of the looping of a pair of chromosomes that are already split lengthwise. *A*, two threads making one complete twist; *B*, the inner strands of each thread interchange at the crossing points; *C*, the threads next flatten against each other, still keeping their twisted configuration; *D*, just before, or after the conjugated thread enters the spindle it opens out by a reduction split that takes place in each segment of the thread. If the strands still keep their spiral relation, two strands cross over at each node; at the ends it is the non cross-over strands that cross, in the figure, but it is the other strands in each case that have broken and "crossed over." The crossed strands in the figure are not due to perspective.

The rings in two planes, as represented in Janssens's diagram, call for further analysis. We may call these rings *Bb*, *Cc*, *Dd*, *Ee* (Fig. 1 *A*). It will be observed that

in ring *Bb* the dark double thread (half ring) at the right separates from the light double half at the left. This is a reduction division for this segment. On the other hand in the ring *Cc* the division plane separates equationally the halves of the dark and of the light half rings. Crossing over takes place at the node between ring *Bb* and *Cc*, and at the node between the rings *Cc* and *Dd* there is another crossing over between the other two strands. Generalizing the result it may be said that crossing over of two of the strands takes place at each node. In the second division, that is supposed to take place here in the plane of the paper, there is assumed to be no further crossing over in either of the halves that have resulted from the first division.

Janssens points out that on this new scheme there is only half as much crossing over as on the scheme represented in our older diagram (1913); but it is obvious that this is only because in the latter whole chromosomes (each potentially or actually made up of two strands) are represented as crossing over at each node. If, however, we compare this latest scheme of Janssens with the figures that we have now recently published ("Physical Basis of Heredity," 1919) in which, following some of Janssens's earlier diagrams only two of the strands cross over at each node, it is perfectly clear that these later schemes of ours give the same number of crossovers per complete twist as do Janssens's present diagrams.

It may, therefore, not be without interest to compare Janssens's latest scheme with the one I have recently suggested in my book on the "Physical Basis of Heredity" (p. 105) where a figure is given that suggests an explanation of the opening out of a twisted conjugated thread in rings that lie in different planes. This figure is here reproduced, Fig. 8 *A–D*, modified only so far as to make it comparable with Janssens's new diagram. In *A* the two split threads are represented as looping or overlapping in an open spiral (an earlier stage than

Janssens's first figure). At this stage where the inner strands come into contact they are represented as fusing with each other at three nodes. The threads may next be supposed to flatten against each other to make the conjugated threads keep their spiral configuration and then condense to make the thick threads. In this condition they pass to the equator of the spindle or they may begin to open out before they reach the equator, Fig. 7 *B*.

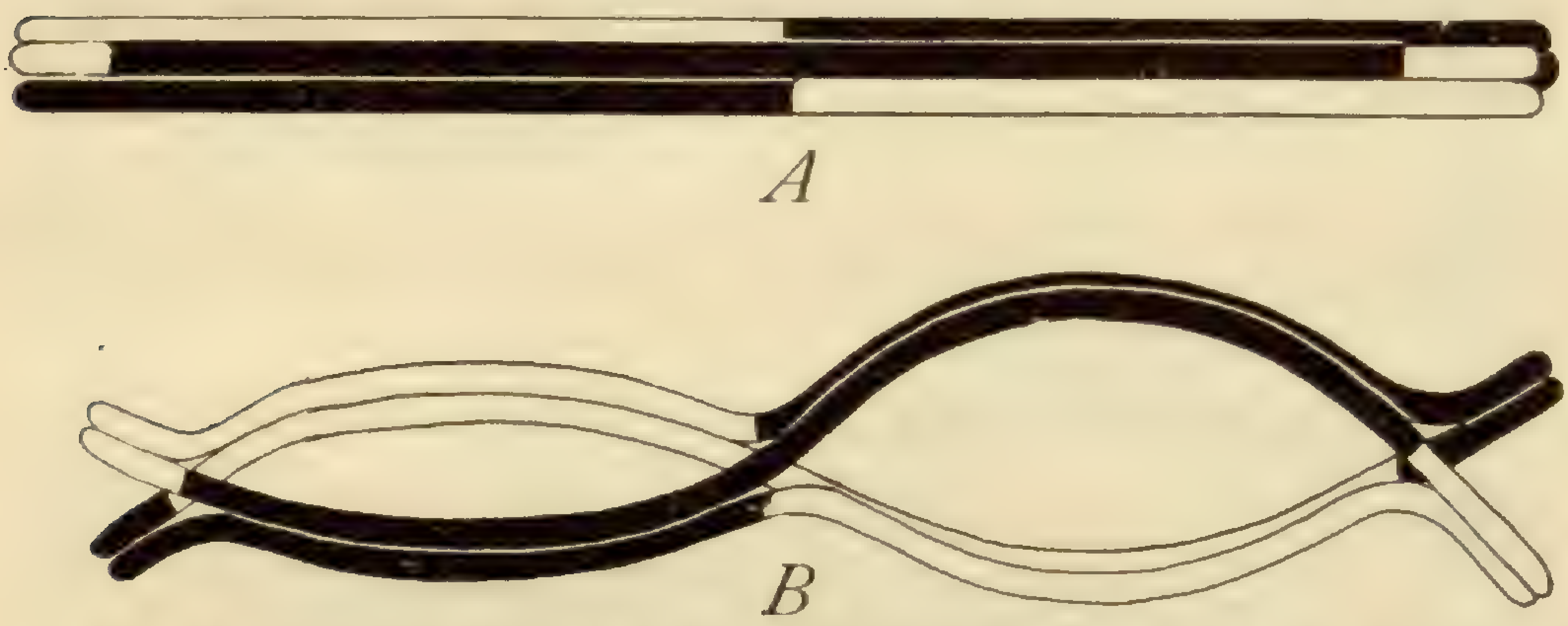


FIG. 8. Diagram showing how the twisted strands of Fig. 8, *C*, become straightened out (untwisted) as the thread shortens, so that the former spiral relation is lost. The resulting relation of the threads when they open out by the reductional separation is shown in *B*. *A*, this figure shows the relation of the strands when the spiral in Fig. 8, *C*, untwists, *i.e.*, as the thread shortens; *B*, if the thread shown in *A* now opens out by a reductional separation in each segment, the resulting figure, *B*, is the same as that of Janssens. In the middle of the figure it appears as though two "cross over" strands were crossing. This is here due to perspective.

If the first division is reductional for every part of the thread, the halves of the thread move apart in opposite directions, and as a consequence of the way the twisted threads have flattened against each other this opening out may produce rings lying in different planes, Fig. 7 *D*, not necessarily at right angles to each other, but at an angle with each other.

The rings are assumed to be due to the reductional separation of the segments of the chromosomes along the tetrad, but the further movements of the daughter chromosomes after they have reached the equator of the spindle must be referred to another mechanism that now comes into play, namely, the forces that carry the chromosomes to the poles. Under these circumstances the

threads may be thought of as separating without assuming such a strictly symmetrical form as Janssens's new diagram indicates, or in other words the separation of the chromosomes may take place as Janssens described it in *Batracoseps*. The suggestion that I made to account for the appearance of rings in different planes was made to meet an objection raised by Robertson and by Wenrich, namely, that the crossed threads (the chiasma threads) do not mean that crossing over has taken place in that region. They point out that the crossed threads may mean no more than that a *not-twisted* tetrad has opened out in different planes in consecutive regions. This obviously may be the interpretation of the crossed threads, but if as I suggested the opening out of the rings themselves in different planes represents consistently a reductional separation in a formerly *twisted thread,* then the cross threads come to have a meaning, for they represent the level at which an earlier fusion and reunion of the inner strands of the four strand stage took place. From this point of view the cross strands, while having nothing to do *at this time* with crossing over, nevertheless correspond to levels at which that process occurred.

I do not wish to appear to be advocating the scheme that I suggested as the best or as the only one that is involved in crossing over. Any scheme that accounts by means of twisting threads for interchange between the segments of homologous chromosomes will fulfill sufficiently the present requirements of crossing over. Much more cytological and genetic work too will be necessary before it is possible to state when and how this process goes on. One point alone seems at present to be indicated with some probability by the genetic evidence, namely, that it would appear simpler for the interchange to take place when the lines of genes are extended to the fullest extent possible, and this would seem most easily to take place, in the accurate way indicated by the genetic facts, when the leptotene threads have spun out to their farthest extent. Whether Janssens also ascribes to this

stage the essential step in the breaking and reunion of the strands remains to be seen when his new results are published.

Until Janssens publishes a full statement as to how he supposes the crossing over at the nodes to take place, whether at the time when the looping of the threads is present, or at an earlier stage, it is hazardous to make too detailed comparisons, but one relation should not pass unnoticed. In Janssens's figure four rings seem to be involved in one complete twist of the two chromosomes. In order to place these rings in such a position that a single (vertical) plane can sunder successive rings transversely and longitudinally in alternation, the rings must be turned so that two are exactly vertical and two are horizontal. A spiral relation of the threads can not be brought into this relation unless the threads first untwist. How this can be done is shown by a comparison of Fig. 7 with Fig. 8. In Fig 7 *A*, as explained, two chromosomes, each of two strands, are represented as looped around each other in an open spiral. In the middle of the spiral the two inner strands that touch are represented as fusing and reuniting to give the cross-over, and near the ends, where the threads cross again, the other two strands fuse, break, and reunite to cross over, Fig. 7 *B*. The threads are then represented as flattening against each other, still keeping their spiral configuration. When they open out again, by a reductional separation of the segments, Fig. 7 *D*, the rings are formed, and if the threads are still represented as keeping their spiral configuration no single plane, as explained, will separate them without cutting some of the strands. But if when stage *C* is reached in Fig. 7 the threads straighten out as they condense (*i. e.*, if they untwist) the result will be that shown in Fig. 8 *A*. If now the threads open out by a reduction division in each segment, the resulting figure will be like that shown in Fig. 8 *B*. This figure is the same as that of Janssens, and the halves can be separated in one plane, as he explains. We may conclude then, if the con-

jugated threads after crossing over do not untwist, they will give figures like those in Fig. 7 *D*, and such threads must be pulled apart as Janssens has explained for *Batracoseps*; but if after crossing over the twist is rectified as in Fig. 8 *A* the threads can separate as Janssens explains for the grasshoppers. In both cases the crossing over is represented as the result of twisting threads, and if such loops tend to have a modal length, the mechanism furnishes a beautiful explanation of interference which is one of the crucial tests to which our explanation of crossing over has been put.

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# VARIATIONS IN THE SECONDARY SEXUAL CHARACTERS OF THE FIDDLER CRAB

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IN species in which the ordinary individuals are sharply separated into males and females there are occasionally found abnormal individuals in which characteristics of one sex are mixed with those of the other sex. We are only at the beginning of the study of these cases, but enough work has been done to make it more than probable that there are several, or even many different kinds of situations that call for separate treatment. That this is generally becoming recognized is evident from the different names that have been used in describing these cases, such as intersexes, sex intergrades, hermaphrodites, gynanders, androgynes, pseudo-hermaphrodites, free martins, eunuchoids, protandrous hermaphrodites; monœcious, diœcious, triœcious plants, indifferent larvæ, neuter insects, etc. It seems to me not worth while at present to attempt to classify such material until we have learned more about it. Whether all or only some of the aberrant types of fiddler crabs here described should be called intersexes depends largely on the definition of what that term includes.

In the fiddler crabs the male (Fig. 1 *A*) and the female (Fig. 1 *B*) show not only the characteristic differences of other decapods, but one of the claws of the male is enormously enlarged. It may be either the right or the left one. If removed a new claw of the same kind regenerates from the stump although it may take more than one molt for the claw to become as large as the one removed. In the fiddler "compensatory regulation" does not take place as in some other decapods (Alpheus); that is, after removal of the large claw, the smaller one does not enlarge and substitute for it at the next molt.

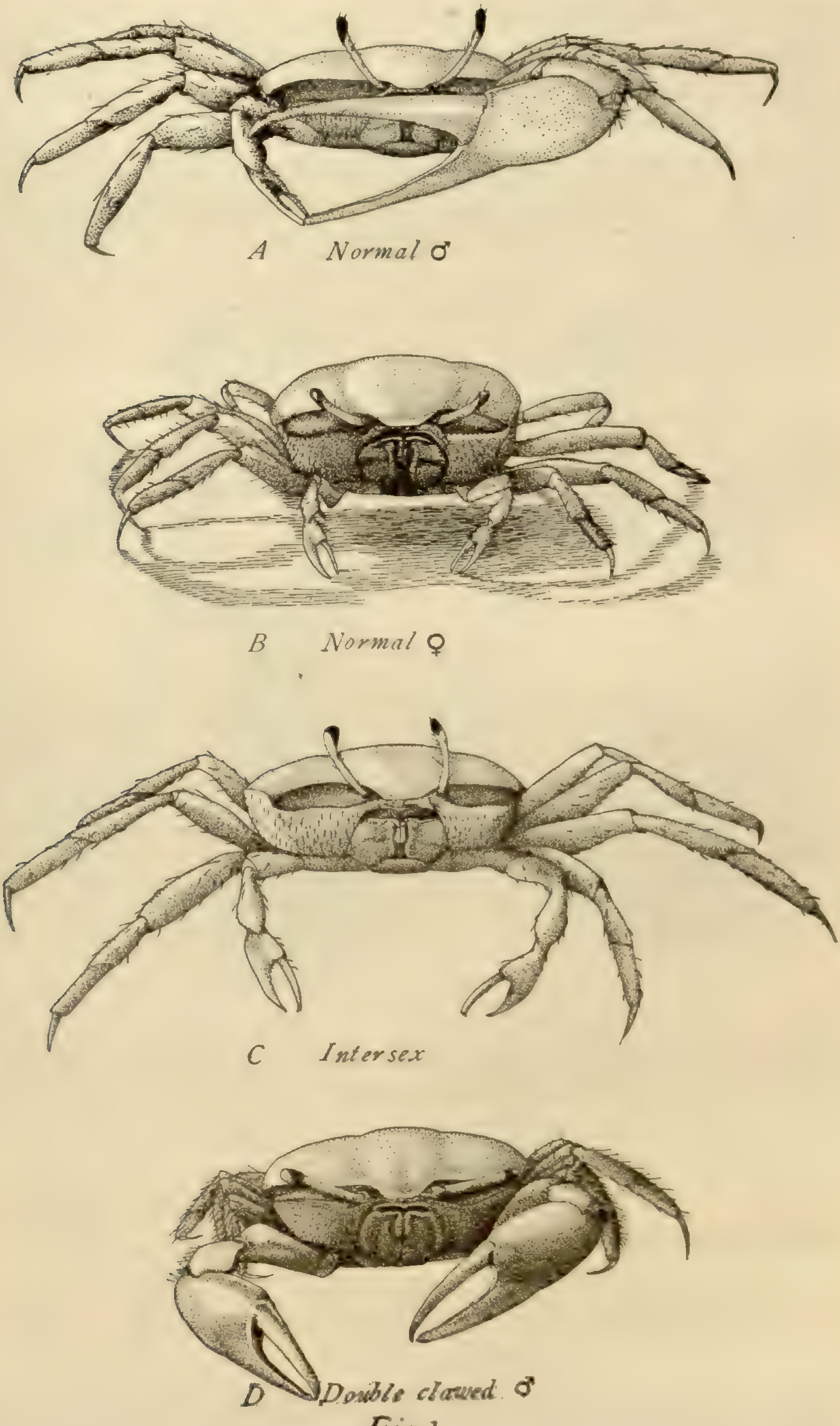


*Fig* 1

Moreover, and this is important, the characteristics of the new big claw are apparent as soon as the regenerated part begins to take shape, and even long before the molt.

The other most characteristic difference in the external parts between the male and the female is found in the abdomen. In the male, Fig. 2 *B*, it is narrow, in the

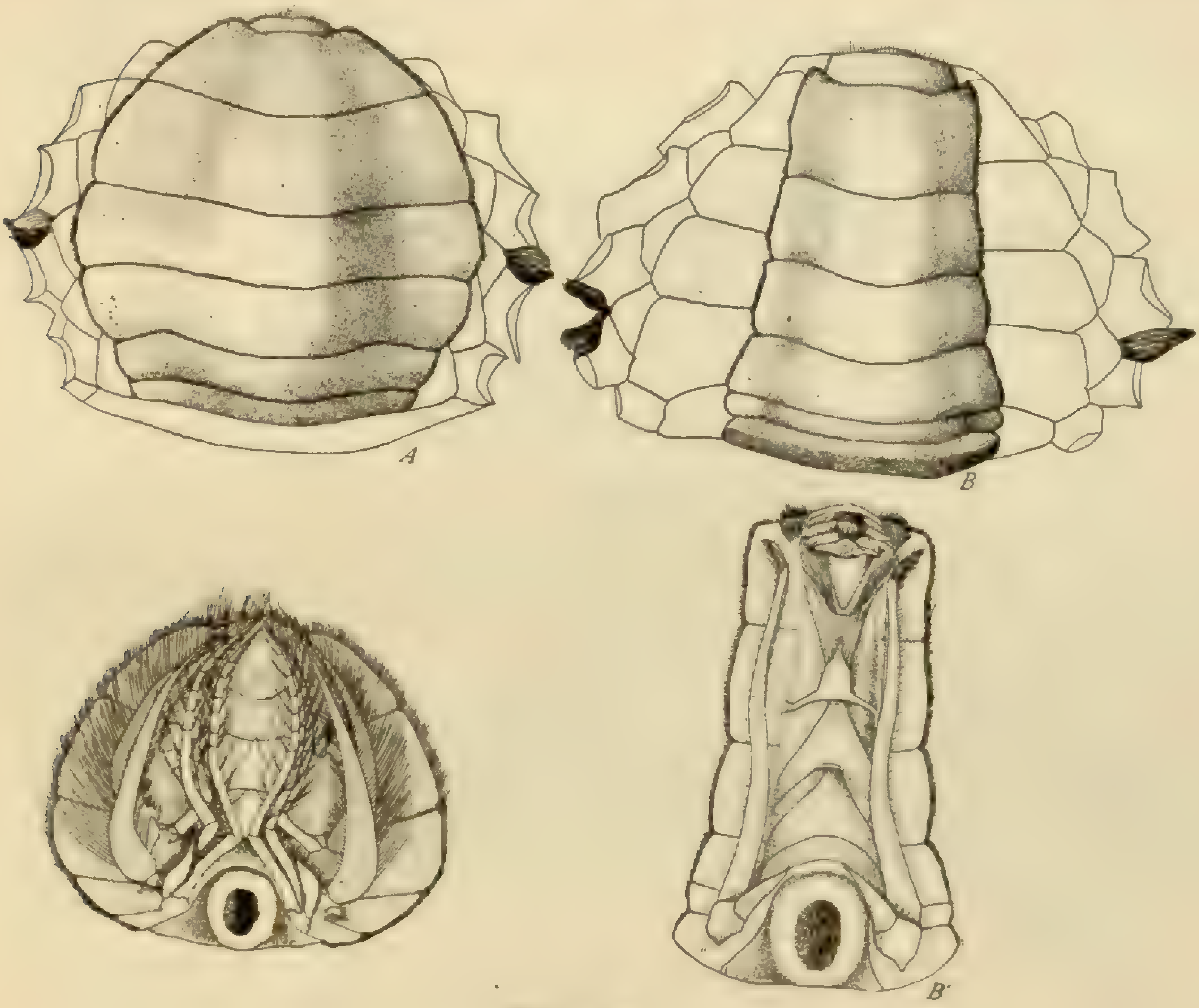

*Fig 2*

female, Fig. 2 *A*, it is almost as broad as the ventral surface of the thorax against which it is plastered. If the abdomen of the male is lifted up, its anterior pair of abdominal appendages, modified into copulatory organs, can be seen (Fig. 2 *B′* and Fig. 3 *A*). In the female the abdominal appendages (Fig. 2 *A′* and Fig. 3 *B, B′*) are entirely different, and are used to carry the eggs. The external genital pores can also be seen when the abdomen is lifted up; those in the male on each side of the middle line are in the segment that carries the last (5th) pair

of legs, in the female they are further forward on the segment that carries the third pair of legs.

In the summer of 1917, at Woods Hole, Miss Grace Hays, while sorting out some fiddlers, found an individual that had a small first pair of legs like those in the female, but an abdomen like that in the male. Another collection of crabs was made and three other such individuals were found. In the summer of 1919 the collectors at Woods Hole brought to me more fiddlers, and from them more aberrant forms were obtained. It then

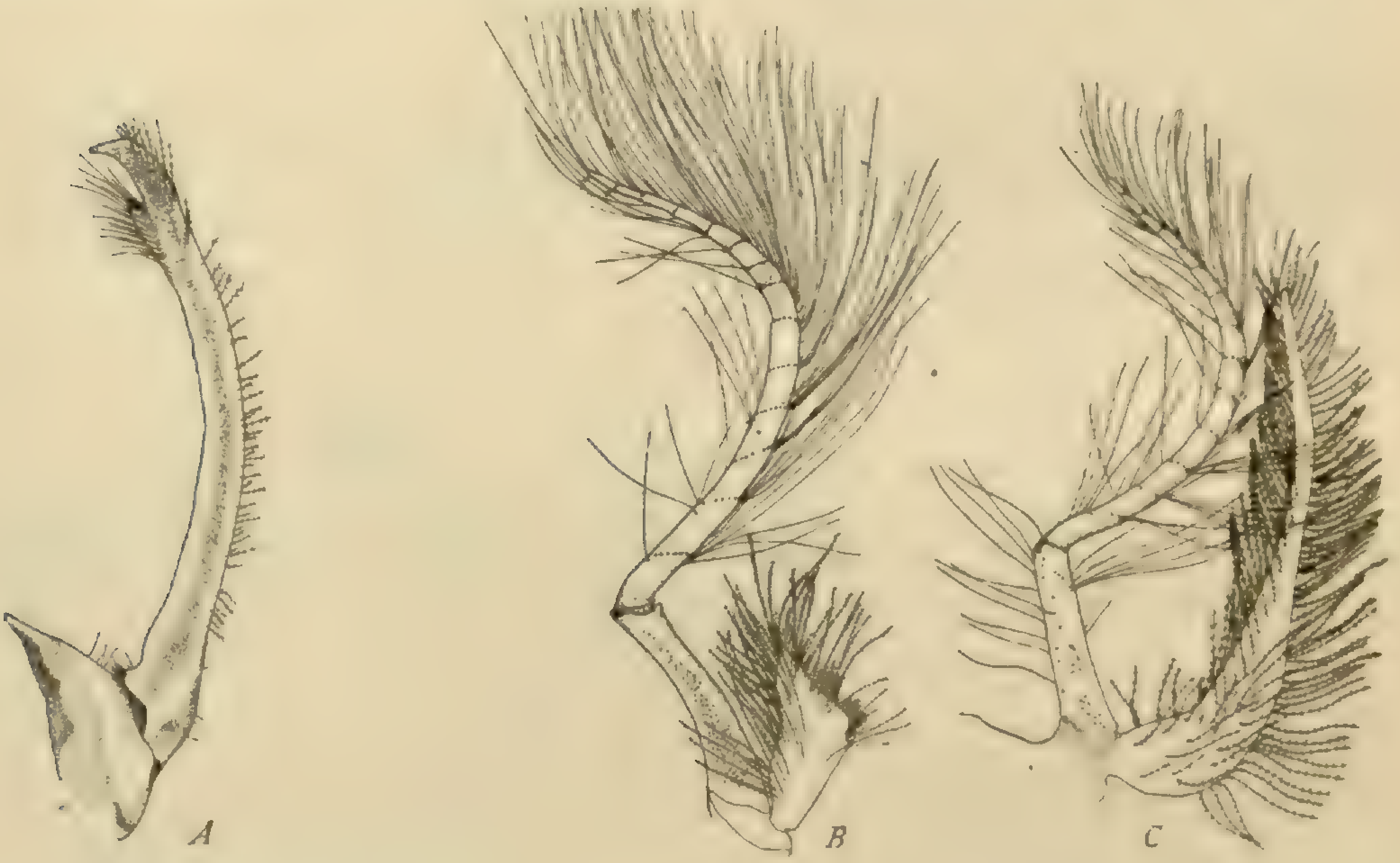


Fig 3

seemed worth while to find out how often such individuals occur in this locality. Thanks to the interest shown by Mr. Wm. Procter and Mr. Alfred F. Huettner a large number of crabs were collected and carefully looked over.[1] As shown in the following table the number of aberrant individuals was found to be about .0077 per cent.

| Normal | "Intersex" | |
|---|---|---|
| 353 | 5 | June 24, 1919 |
| 50 | 2 | 28 |
| 1,245 | 6 | 30 |
| Total 1,648 | 13 | |

[1] A few small intersexes were later obtained by Mr. Lionel Strong at Cold Spring Harbor, L. I. Mr. Procter later collected 2,068 crabs at South Wellfleet (July 4), but found no aberrant individuals amongst them.

A cursory examination showed that two types of individuals were present. The larger individuals had the abdomen of the male sex, but both claws were small and

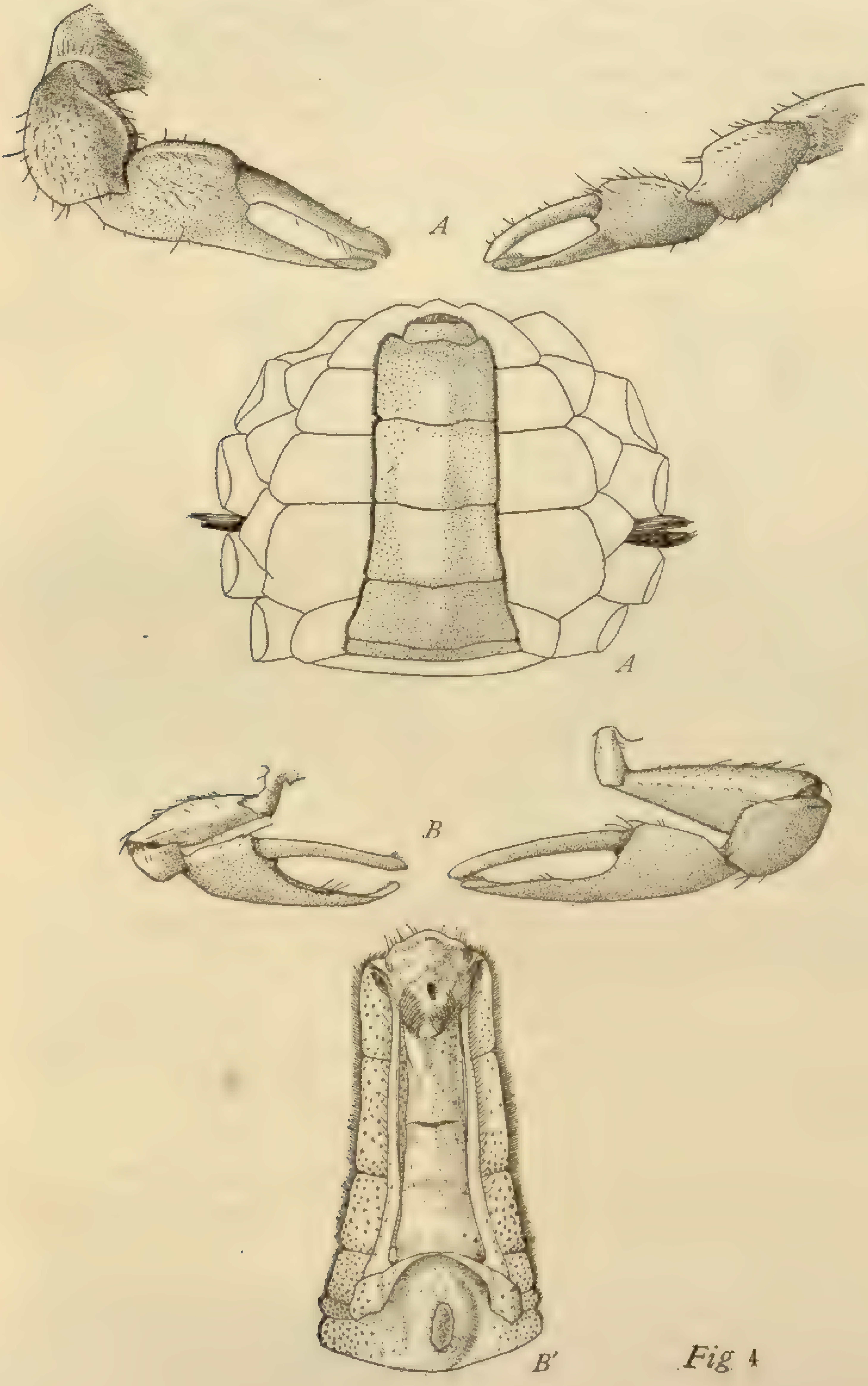


*Fig* 4

more like those of the female (Fig. 1 *C*). The other individuals had abdomens not quite so broad as those of typical females of the same size, Fig. 4 *B′*, yet their claws were generally small, like those of the female, and showed no indications of a variation towards the male type. Since the younger stages of some crabs, such as the blue crab, have a narrower abdomen than that of the adult female until the last molt, it seemed possible that these "intersexes" might at a later molt turn into typical females. They were kept therefore and well fed for two or three months during which time they molted once, Fig. 4 *B″*, or even twice. A comparison of the old skin showed that the condition of the abdomen and claws had not changed. It is evident that this condition can not be explained as transitory. Nor is it juvenile because normal individuals of the same size have the abdomen full width.

A more detailed examination of these two types may now be given. The most striking fact is that all of the full grown crabs belong to one category, and all of the smaller ones to another. The former of which there are six, have a strictly male abdomen regardless of the condition of their claws, and what is more significant the external genital pores are at the base of the last pair of legs, as in the normal male. As shown in Fig. 2 *A′* and *B′*, the abdomen is exactly like that of the male. On its inner side it contains the two long copulatory appendages of the male. The chelæ in three individuals are small, Fig. 4 *B*, and of the same size, resembling those of the female. In the other three one of the claws is somewhat larger, Fig. 4 *A*, than the other and shows unmistakably evidence of variation toward the male. The genital pores are, as stated, in the same position as in the normal male, and there are no indications of female pores further forward. In two cases at least, the crabs molted, but did not change their characters. In the second group, Figs. *A*, *B*, *C*, *D*, there are sixteen individuals. There is amongst these no obvious relation between the size of the

crab and the relative width of the abdomen. Some of the smallest have the narrowest abdomen. There is some correlation between the character of the abdominal appendages, particularly the first pair and the width of the abdomen. As shown in Fig. 6 *A*, *B*, this appendage does not appear as much like that of the female of the same size, Fig. 6 *C*, as would be expected were it strictly female, yet it can not be said to be male-like, and the narrowness of the abdomen may be responsible for the difference.

The claws are like those of the female in all cases.

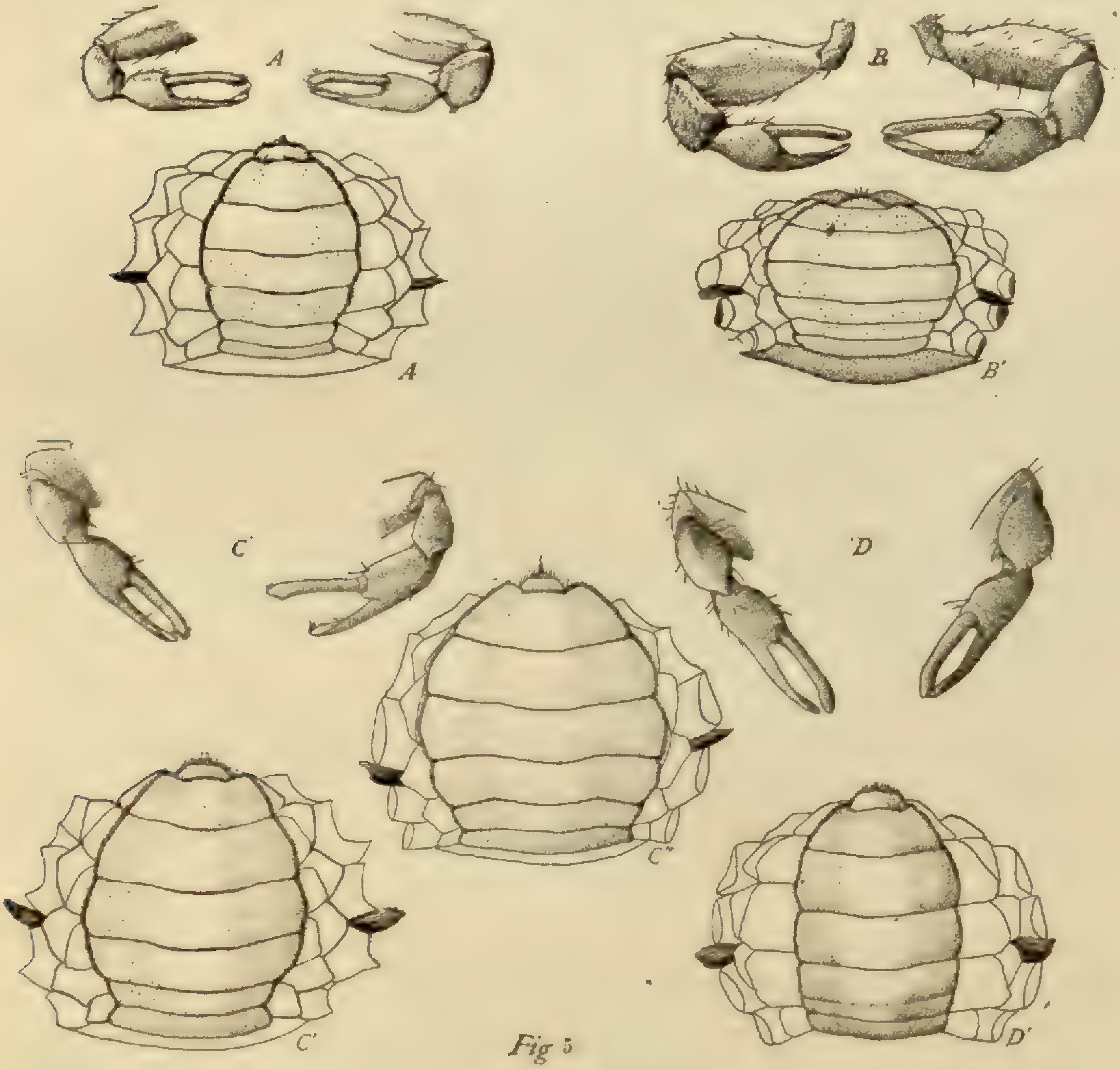


Fig 5

After the foregoing account was written I have received from Miss Rathburn a number of fiddler crabs, exactly like those recorded above, from the collection in the National Museum in Washington. They fall into the same

two groups. One large male, labelled *Uca pugilator* (18286), has a pair of small female like claws. It comes from Northampton Co., Va. (1894). It belongs to a different species from those described above.

There are in this collection eight small female-like crabs with the abdomen narrower than that of the normal female of the same size. All of them have both small claws of the same size. In those with the narrowest ab-

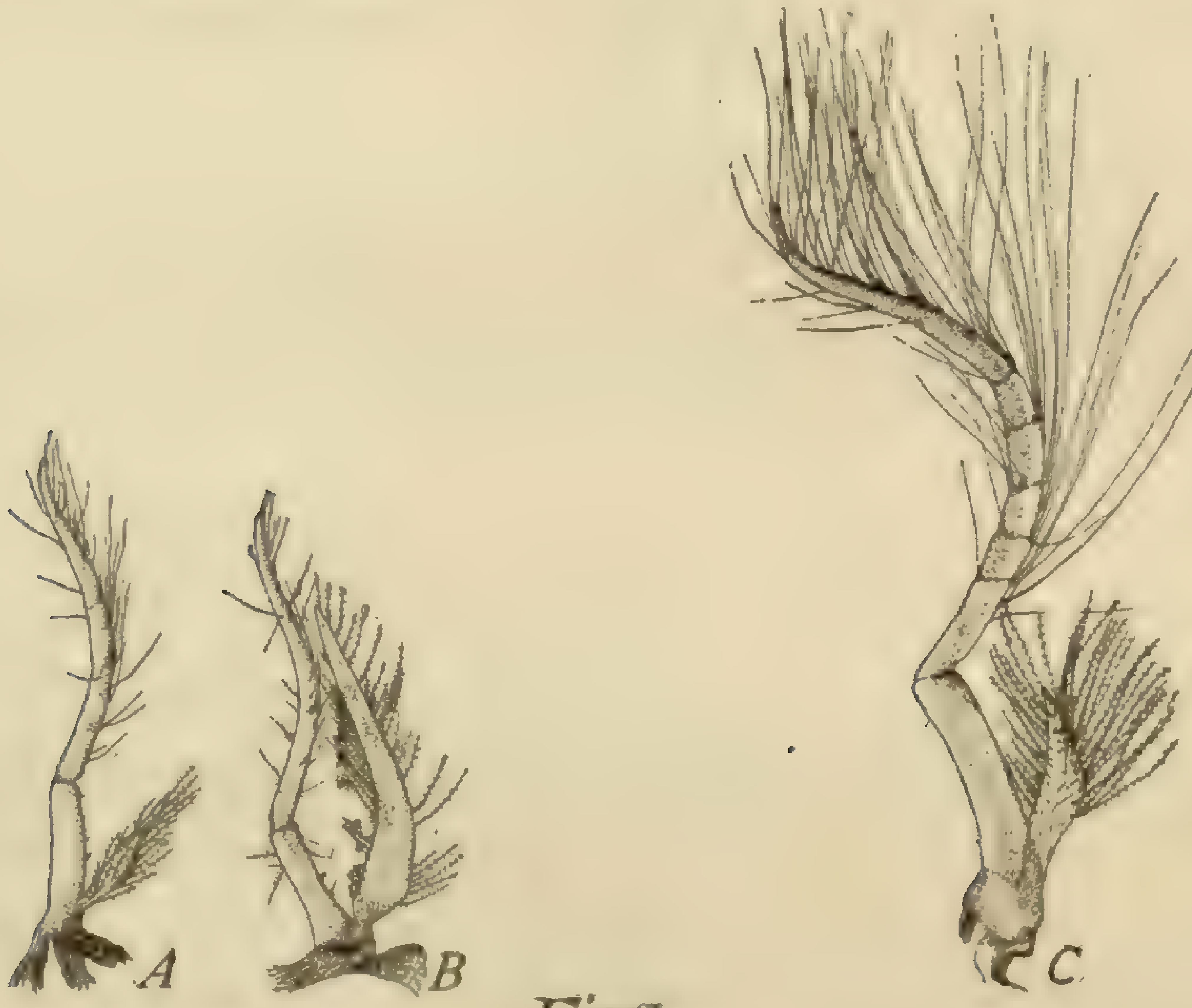


Fig 6

domen, the abdominal appendages are straighter and less plumose than are those of the normal female of the same size. This condition might be described either as a juvenile, or as a less female-like condition, but not necessarily more male-like. In size and shape the abdomens of these crabs are like those of Fig. 5 *A, B, C, D*. In addition there is one small individual (17688) labelled "pugilator," with the abdomen about half the width of a normal female of the same size of the other species.

There is also one further variation in the fiddler that is different from the preceding ones. It is shown in Fig. 1 *D*. Mr. G. M. Gray found this male fiddler (*Uca pugilatur*), and with his permission I am able to figure it here. It had two large claws, both like the claw of the normal male. I find that Professor S. I. Smith, of New Haven, recorded in 1869 a similar case of *Uca pugnax*.

At present we are entirely ignorant as to what causes determine in the normal male that only one side develops a big claw. The asymmetry of the fiddler appears to be analogous to that of the asymmetry of snails and of the one-sided operculum of certain annelids (*Hydroides*), etc. It is generally supposed that something comes in during the development of the male crab that turns the scale one way or the other; and once determined the relation persists during life in fiddler crabs, although in other decapods, as shown by Przibram, the initial difference may be reversed during regeneration if the large claw is removed and the small one left. Until we get further information concerning these matters it would be idle to speculate as to what has led in this male to two large claws.

It is interesting to note in the case of this male with two large claws that it differs from the ordinary males by doubling the kind of difference that distinguishes the normal male from the female. It can scarcely be said to be an inter-sex, for the difference is not in the direction of the opposite sex, but away from it. If some designation is called for, it might be said to be a super-male, or at least an over-clawed male.

### Discussion of the Results

If we compare the results of parasitic castration of certain decapods with the conditions described here in the fiddler crab several resemblances and differences become apparent. First in none of the cases of parasitized crabs are the external genital openings affected. They furnish a certain clue to the original sex of the individual. Likewise in the large fiddlers the male external genital pores are present, and there are no female pores. The individuals have probably always been males. Whether the condition of their claws is due to some disease, or possibly to some internal parasite, or to a change in the genetic complex, can not be stated. It is even possible that it may be due to none of these, but to some "accident" in the development, *i. e.*, to some change in the em-

bryology that determines the asymmetry of the normal male. The occurrence of the male with the two large claws may seem to favor the last interpretation; for here we find the reverse relation and it does not seem probable that such an over-clawed male could have arisen through parasitism, or through disease, although the argument for a genetic change can not be entirely set aside.

In regard to the other group containing the small aberrant forms the situation is somewhat different. All of these have started as females, as the location of the external genital pores clearly indicates. Yet some of them show also an apparent change towards maleness by the narrowing of the abdomen or possibly a retention of the juvenile condition. Here the change, if it be a change, is in the reverse direction from that shown by most of the intersexes described by Giard and by Geoffrey Smith, since starting as a female the change is towards maleness, while in the parasitized crabs it is the female that changes towards the male. The different degrees to which the change has taken place in different individuals may seem to indicate disease or parasitism. The absence of further change in the same direction in the next or following molts is not perhaps so favorable to this interpretation. But on the other hand the absence of adult crabs of this sort, or at least their infrequency may mean that these individuals do not reach maturity, and may therefore be diseased or infected[2] or that in the adult the full-sized abdomen is attained. These questions must be further investigated before a decision can be reached.

## General and Hypothetical

It may seem, as stated above, that some of the changes seen in the fiddlers may be similar in kind to some of those brought about in other crabs by becoming parasitized. Giard has described several cases in crabs and

[2] Geoffrey Smith has described changes in the crab *Inarchus* brought about by inflection of a gregarine. The abdomen and claws of the male were changed in much the same way as when this crab is parasitized by a barnacle. Giard has described an hermaphrodite amphiurian, parasitized by Orthenectidæ, that cause the ovary to degenerate while the testes continue to function.

other Decapods parasitized by other crustataceans (*Sacculina Portunion, Peltogaster,* etc.), in which changes take place in certain parts of the body that approach the condition found in the opposite sex. These changes involve most often the abdomen and its appendages, and in one species at least the claws. The most marked changes involved the male, producing in him alterations in the direction of the female. In one case *a female* is described as showing some effects of the parasite but if I am correct Giard interpreted this change as resulting from the retention of the juvenile condition. Giard contrasts the changes in the male crabs with those produced by castration in the vertebrates. He seems to imply at times that he supposed the effects are produced by the loss of the gonads. At other times, however, he speaks definitely of the changes as some sort of symbiotic relation between the host and the parasite—an idea similar in many respects to the later and more elaborated hypothesis of Geoffrey Smith. In fact, in summing up the evidence Giard recognizes two classes of cases; those due to the indirect action of the parasite by way of the testes, and those due to the direct, by action on the host. It was, of course, at that time natural to suppose that in both groups, vertebrates and crustaceans, castration acts in the same way, especially as the case of the vertebrates had been long in the literature, and zoologists had become familiar with this kind of effect. Moreover at the time other evidence was lacking to show in other groups that the gonads have no influence on the development of the secondary sexual characters. But the work of Oudemanns that was later fully confirmed and extended by Kopec and by Meisenheimer and by Kellog removed any prejudice that the situation in the vertebrates had brought about, so that at the time when Geoffrey Smith wrote the field was clear for an independent judgment. As stated Geoffrey Smith brought forward evidence that seemed to him to show that the changes in the secondary sexual characters in parasitized males were due to physio-

logical processes set up by the parasite in the host. He even went so far as to compare directly and in detail the substances called forth in the host by the action of the parasite. Without discussing these questions here (since I have recently discussed them in my paper on "The Genetic and Operative Evidence Relating to Secondary Sexual Characters," Carnegie Publication No. 285, 1919) it is evident that crucial experiments must be made on the crabs themselves before a conclusive case can be made out. This is by no means a simple matter as I have found. During the last three summers at Woods Hole I have tried to carry out experiments on crabs to test some of these questions. All attempts to remove the gonads in fiddler crabs have failed, because of the delicacy and distribution of the organs, and the fatalities that result when the carapace is lifted up. Attempts such as Stamati made in 1880 to destroy or injure the gonads by injecting substances through the genital pores have also failed, because of the delicacy of the tubes and the distance of the gonad, in the male, from the external genital opening.

Some important observations made by Kornhauser (1919) on the effects of parasitism of the tree-hopper, *Thelia bimaculata,* by the hymenopteron, *Amphelopus thelia,* have a bearing on the preceding discussion. The egg of the parasite is deposited within the body of the nymph of *Thelia* from the first to the fifth instar. The egg or eggs give rise to a number of "polyembryonic" larvæ, that ultimately destroy the host. Infected males show in the adult stages many of the characteristics of the female, the degree to which the change takes place being mainly dependent on the stage at which parasitism occurred. The change involves the pigmentation, the size, certain abdominal spines, the shape of the abdominal sclerites that approach or even reach the condition found in the female. The genital appendages do not change into those of the female, but remain small and lose their specific characteristics.

Parasitized females do not assume any of the features peculiar to the males.

The gonads in both sexes usually degenerate, and an accumulation of fat takes place in the abdomen of the host. Two exceptional cases have an important bearing on the cause of the changes resulting from the parasitism. One male was found that had been parasitized, and although it had been considerably changed towards the female in its somatic characters it "contained full-sized normal testes with many spermatozoa." Evidently then the changes caused by the parasite are not due directly to the destruction of the gonads as shown by this individual in which the gonads had escaped. This accords with the results of artificial castration in other insects.

The other exceptional case (fourth instar) had a "perfect female soma" but contained testes. The individual had started as a female. There was evidence of this, though it is not conclusive, in the chromosome counts of the somatic cells. It must be supposed that at an early stage something changed the cells of the germ-track, so that its cells developed into testes. This conclusion is borne out by a count of the chromosomes of the testes that show 21 cells in the spermatogonia, one of them being the large *X* chromosome characteristic of the male. An early "elimination" (loss) of an X chromosome from the mother cell of the germ-track, such as occurs in *Drosophila,* would seem to be the simplest explanation of this case, as suggested by Kornhauser.

The conclusion from the evidence is quite convincing, namely, that the several characters peculiar to the male are changed into those peculiar to the female as a result of the direct action of the parasite, and not through any influence by way of the gonad.

### FEEDING FIDDLER CRABS ON THE GENITAL GLANDS OF THE OPPOSITE SEX

During the summer of 1918 I carried out some feeding experiments. The occurrence of hormones in the repro-

ductive "glands" in other animals suggested the possibility that the secondary sexual characters of fiddler crabs might be affected if the crabs were fed on the organs of the opposite sex during the period of regeneration of the large claw. Male fiddlers whose large claw had been previously removed, were fed exclusively, and at intervals of two or three days, on the ovaries of female spider crabs. The fiddlers were kept until they moulted about a month or two later. The new claw showed all the characteristic features of the normal large claw. Its regeneration had not been affected by the character of the food.

Female fiddlers, one of whose claws had been previously removed, were fed on the testes and the ducts leading from them of the male spider crab. Other females were fed on what appears to be a large gland in the posterior part of the abdomen of the male. No effect on the regenerating claws were observed.

These negative results do not show that there is no hormone in the gonads of the crab that affects the secondary sexual characters, for even if there were such, it might not be able to produce its effect through the digestive tract. Only positive results of this kind would be important but none were obtained. As the results were entirely negative they need not be further described.

A more promising test consisted in boring a hole in the carapace of the male fiddler and inserting pieces of the ovary of the female fiddler. Conversely for the female. There are certain implications in Geoffrey Smith's views that seem to imply that male tissue can not survive and grow in an individual with female metabolism, and perhaps conversely for the male. The small grafted pieces often become lost, and it is difficult to determine later by means of sections how far the tissues degenerate and how far they become implanted and grow. I have not had the time to carry out a detailed study of the sections, but they seem worthy of further examination. No effect on the claws were produced.

## A Piece of an Ovary Present in a Male Crab

A normal male fiddler was opened to obtain pieces of its testis. In the region where the left testis is supposed to end there was found a small piece of ovary with its purple eggs. These were sectioned and found to be normal eggs. This observation is significant in so far as it shows that ovarian tissue can grow and differentiate in a purely male environment. The explanation of this occurrence is not at hand. One is tempted to refer to it an abnormal cell division in the testis of such a sort that the chromosome combination (if such exists) to produce eggs was formed, but in the complete absence of information concerning the chromosome composition of the male and female crustacea, such an attempt would appear premature.

## Intersexes and Gynandromorphs in Crustacea

If it does not seem probable that the aberrant types in the fiddlers, that have been described above, can be safely referred to parasitism, it may not be without interest to point out that there are many other queer cases of sex mixtures in the crustacea, that do not appear in any way connected with environmental changes—or at least not directly.

Many cases of intersexes have been described in the Cladocerans, in the genera *Daphnia, Alona, Leptodora, Simocephalus,* under the name of androgynes, gynandromorphs, intersexes, etc. (Kurz, 1873. Grochowki, 1896. Woltereck, 1908. Kutner, 1908. Ashworth, 1913. Agar, Banta, 1918. De la Vaulx, 1915 and 1918). The antennæ more often show modifications characteristic of both sexes, but other organs are frequently involved, including even the gonads. There are no indications of parasites in any of these cases, where owing to the transparency of the body they would be easily detected if present.

Kutner has recorded the sporadic occurrence of intermediate forms through 12 generations of *Daphnia pulex* —in a line having a relatively high percentage of these

forms. No recognized form of inheritance can be detected in these parthenogenetic lines. If, as generally supposed, there is no elimination of chromosomes in the parthenogenetic egg of Daphnians, the expectation would be that all offspring of an intermediate would be like the mother, whether the "character" were recessive or dominant. It would seem then that if certain lines of parthenogenetic Daphnians do produce more intermediate types than occur in the general population, we must look either to irregularity in the chromosome behavior or to environmental influence. The latter seems excluded by Banta's results to be mentioned later. The former can only be hypothetical until such differences are found. Nevertheless the discovery of such cases in other groups (*Drosophila, Œnothera*) makes the suggestion at least not so speculative as might have appeared several years ago.

The most important results are those recorded by Banta, not only because he has obtained a much higher percentage of intergrades, but because these appeared in a pedigreed strain, and the appearance of the intergrades has been carefully followed through later generations. In the 131st generation of one of the strains there appeared males, females and *sex intergrades.* The last group composed of "males with one or more female secondary sex characters, females with one to several male characters and some hermaphrodites with various combinations of male and female secondary sex characters." Highly male-like females produce only a few young or are sterile. "A female intergrade with as many as six strong male secondary characters rarely produces young." Males that have one or more female characters have nearly always incompletely formed testes. The strain was kept up by breeding from female intergrades that continued to produce females, males, and sex intergrades for 16 generations with no apparent change in the ratio of the various sex forms." The picture here presented can not but suggest some sort of disintegration or

variation in the chromosome mechanism. At present we do not know how a parthenogenetic female sometimes produces female (parthenogenetic) broods, at other times male broods or sexual eggs. That such changes may be brought on by environmental changes seems not improbable from the large amount of data already collected. The results in these respects are so similar to those in rotifers where the situation is now under control (Whitney) that one can scarcely resist the conviction that in both cases the environment acts in producing the changes. But while we have no explicit evidence, as yet, even in *Hydatina,* that the environment acts only by bringing about changes in the chromosome mechanism, there is at least nothing known opposed to such a view, and some general arguments that incline one to anticipate such a discovery. Until these matters are set straight not much is to be gained by speculating as to how the sex intergrades of *Simocephalus* and other *Daphnia* arise. But if it should be found that the normal cycle is caused by alterations in the chromosome cycle, as has been shown in fact for Phylloxerans and Aphids, then I think we may have to look to some aberrations in the same mechanism to explain these anomalous cases. Indeed the kind of inheritance described by Banta appears to be one that might be expected from such a situation.

In the genus *Cyclops,* Mrázek (1914) has described "androgynes" that have modifications in the antennæ, and Bremer (1914) has recorded two cases of "pseudohermaphrodites" in *Diaptomus.*

In sharp contrast to these kinds of intersexes in the lower crustaceans stand out the bilateral gynandromorphs that have been found in two genera of lobsters. Nichols in 1734 described a lobster whose right side was female and whose left side was male. Dissection showed an ovary in the female side and a testis on the left. A similar case was described for *Palinurus* in 1902 (Burger), but no dissection was made.

In the *Canadian Naturalist* for May, 1919 (Vol. XXXIII, No. 2), there is a description of another "hermaphrodite" lobster. In reply to a letter of inquiry that I sent to Mr. A. P. Wright, he states that the lobster was sent to him by Mr. Halkett and that the animal is male on the left side and female on the right side. There is an ovary on one side and a testis on the other. These three cases appear to differ from the preceding cases and suggest a direct comparison with the bilateral gynandromorphs of insects. It is quite possible that they owe their origin to some similar chromosome "elimination" in the course of development, but it should not be forgotten that sex-chromosomes have not been reported in the lobster, although the cytology of the spermatozoa has been often examined.

Another decapod, *Gebia major,* has been shown by Ishikawa to be hermaphroditic. The anterior end of the testes produces sperm and the posterior eggs. A pair of ducts leads from each part to the exterior. Such individuals appear to function only as males. Spitschakoff found in a crab, *Lysmata seticaudata,* that both ovaries and testes are present with their ducts and external genital pores on the third and fifth pairs of legs. The anterior end of the gonad functions as ovary and the posterior as testes, which is the reverse relation from that of *Gebia.*

In crayfish belonging to the genus *Parastacus,* von Martens, 1870, von Ihring, Faxon, 1898, and Lonnberg, 1898, have described genital pores on the third and fifth pairs of appendages. Lonnberg has dissected some of these individuals. He finds testes in some of them, ovaries in others, but in both cases there are two pairs of ducts lead to the genital pores on the third and fifth pairs of legs. Here there is no true hermaphroditism, but on the contrary separate sexes. Nevertheless the ducts characteristic of the males and females in other species with separate sexes are both present in all individuals of *Parastacus.*

Selbie points out that Wollebaek (1909) showed that *Calocaris macandreæ* is normally hermaphroditic, each individual having testes and ovaries. The first pair of abdominal appendages has its tip expanded in all individuals as in male decapods.

In some of the amphipod crustaceans, the occurrence of ova in young males seems to be a normal occurrence recalling the conditions in frogs. Nebeski (1880) stated that the anterior end of the testis of *Orchestia gammarillus* contained ova. DellaValle, 1893, never found many eggs in *Orchestia deshayesii,* and none at all in sexually mature males. Geoffrey Smith made some further observations and attempted to explain the results on his anabolism-metabolism view. Ch. Boulenger studied the two forms mentioned above. Out of 137 males of *O. gammarellus*, 135 had no ova and 2 had a few ova anteriorly. Of small individuals, on the other hand, nearly all contained ova in the testis (198 with and 19 without ova). "These results are therefore much at variance with those obtained by Smith and I am at a loss to explain how he arrived at his conclusions."

## INTERSEXES AND HYBRIDIZATION

In recent years several cases in which intersexes appeared in considerable numbers have been shown to be due to intercrossing. This raises the question whether some of the aberrant individuals here described may not have been due to crosses between the two species of fiddlers *Uca pugnax* and *Uca pugilator*. It is true that the latter is found most often in sandy stretches and the latter on muddy flats, yet the two are not infrequently found together or in nearby localities. The larger intersexes appear to be unmistakably *Uca pugnax*; the smaller are more difficult to identify. Miss Rathburn has examined both the large and the small individuals here de-

[3] Ewing ('85) has described a blue crab in which the abdomen is intermediate in width between that of the adult male and female. He thinks that the individual is hermaphrodite, but as shown by Churchill, the peculiarity described is the normal condition of the juvenile female before the last molt.

scribed and reports that they all belong to the species *Uca pugnax,* and show no signs of being hybrids.

The most interesting cases of intersexes are those produced by Goldschmidt in crosses between different races of the gypsy moth. He describes some crosses that give individuals showing only a slight tendency towards the opposite sex; other crosses go further until finally the male may be completely transformed into females, the change even including the appearance of eggs in the gonads. Conversely females may be changed towards maleness in various degrees depending on which varieties are crossed. His interpretation in general is that the two kinds of sex genes have different values in different races, so that the hybrids are in these respects betwixt and between so far as the influence of the sex genes is concerned. As I have recently discussed at some length Goldschmidt's view (see Carnegie publication, No. 278, 1919, and No. 285, 1919), I need not go over the ground again.

Harrison has more recently described intersexes in the offspring of different species of moths belonging to the family of Bistonidæ.

In this connection it is interesting to note that some of the phenomena seen in these moth crosses appear when crosses are made between two species of *Drosophila,* namely, *D. melanogaster* and *D. simulans.* Made one way the cross gives only females as A. M. Brown discovered, and as Sturtevant has verified. Reciprocally only males are produced, as I have found, with a few females hatching late in the series. In both cases, however, the hybrid males and females from the two crosses, although sterile, are strictly one or the other sex both in their gonads and in their secondary sexual characters, but as stated the gonads are rudimentary. Sturtevant's recent discovery of real intersexes in a race of *Drosophila simulans* has an important bearing on the interpretation of intersexes. He finds in a certain line that individuals appear that show characters both of the male and of the

female, including especially the genitalia. They have rudimentary gonads. Breeding from normal heterozygous sisters and brothers he has shown that there is present a recessive gene that gives the intersexes when present in double dose in females. This gene is in an autosome. The results are shown, therefore, not to be due to a change in the gene or genes for sex, but to a gene whose effects are superimposed on the influence of the sex genes. It is evident that such a possibility must be reckoned with in interpreting other cases.

Intersexes have been found in human lice, *Pediculus,* by Keblin and Nuttall. The evidence makes it probable that these arise most frequently when the body louse, *P. corporis,* crosses with the head louse *P. capitis.* These intersexes have both male and female gonads and genitalia in the same individual, differently combined.

It has long been known that crosses between Gallinaceous birds give rise to males that are sterile although such males are not described as intersexes. Whether only the male hybrids survive or whether the female hybrids are sometimes turned into males is not known. Guyer has raised the question as to whether the individuals in question if ever females might be classified as males, because of the rudimentary condition of the ovary. It is well known to-day that removal of the ovary of the hen causes her to assume the male plumage (Goodale), and also it is more than suspected that tumors in the ovary or other diseases of that organ produce a like effect on the plumage. But Guyer points out that in the few cases examined by him testes were present. Riddle has described many cases in hybrid doves in which the sexual behavior of certain individuals showed them to have opposite sex tendencies from that indicated by their gonads. These he calls sex intergrades. It is well known to poultrymen that birds in poor condition sometimes behave queerly in their sex relations. It is possible that the weakened condition of these doves may have something to do with their anomalous behavior. But aside

from this question it is possible to state that in one of the crosses at least, in which a sex-linked character is involved, there is good reason to believe that the normal sex chromosome relations persist. It is scarcely legitimate under these circumstances to suppose that the ordinary mechanism of sex production is changed in such cases in the sense implied or stated that males have been turned into females and females into males. Moreover it is sometimes overlooked that if such were the case very anomalous sex inheritance would follow were it possible to breed such hybrids. Unfortunately this is not possible in most of the cases at issue, since the hybrids are sterile, but in the few hybrids that have been bred no such disorder of the machinery appears and the individuals appear to be true to their sex. One must look, I think, in other directions for an explanation of the results.

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# THE SABLE VARIETIES OF MICE

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THE variations in darkness of certain forms of fancy mice have called forth different interpretations from the various investigators who have studied them. The present report is intended as a contribution of experimental data, treating these differences as graded variations.

The varieties of mice most commonly exhibiting differences in darkness comprise those races known as sables. Such mice are distinguished by a yellow belly and a back of some shade of black or brown with which yellow may or may not be mixed. They were first reported by Bateson in 1903 but Miss Durham (1911) was the first to breed them experimentally and to catalog the variations within the sable race. Little (1913) classed sables as yellows with varying amounts of dark pigment in the hairs on their dorsal and lateral surfaces. Dunn (1916) offered the explanation that all sable varieties differed from ordinary yellow by a factor or factors determining the quantitative increase of dark pigments, so that sables formed a continuous series of increasing dark dorsal pigmentation from clear yellow to black-and-tan in which the back was intense black while the yellow pigmentation was exhibited only on the belly. Onslow (1917) was "led to look upon sable as a pattern factor which could give a yellow belly to a mouse of any color," but he did not publish the experimental evidence upon which his conclusions were based. He criticized Dunn for further involving the nomenclature of the sables through the use of the names "black-and-tan," "brown and tan," "black sables," and "brown sables," to designate the members of the sable series.

The use of the above term is, I believe, justified because black-and-tan is recognized by the English fanciers as distinctly different from ordinary sable, and because none of the sables described by Miss Durham behaved as did

the mice used in my experiments. Miss Durham described "black, blue, chocolate and silver fawn mice which differ only from the ordinary forms by having yellow bellies," but they subsequently always moulted into ordinary sables which have a "dark black or brown streak down the middle of the dorsal region while the rest of the mouse is yellow." Black-and-tan however does not moult to ordinary sable. Even at the age of twenty months black-and-tan is entirely black except for the yellow belly and yellow ticking on flanks and muzzle. Since these mice were different from ordinary sables, they were given a name to indicate the difference. Moreover when they were crossed with various non-black-and-tan varieties there were produced in the second generation mice resembling the black-and-tan parent, others intermediate between black-and-tan and yellow, and conforming to Miss Durham's description of sable. The latter were called black sable or brown sable to indicate the color of their non-yellow pigment. I am in sympathy with Onslow's desire to prevent a duplication of terms but I believe that the names employed are required by the presence of types which differ both genetically and somatically.

Sooty yellows may likewise be included in the sable series since these mice appear when black-and-tans or sables are crossed with non-sable varieties. Sooties can not be distinguished simply as yellows which are heterozygous for black, for yellow mice which carry black may show no trace of sootiness. Factors additional to the black gamete are involved in the production of sooties and these factors are present not only in the sooties but in the blacks which they produce. Little (1916) used blacks derived from sooties in crosses with wild agouti and obtained agoutis which were much darker dorsally than any wild agoutis. I have obtained similar results from such crosses. Certain blacks with which yellows have been crossed to produce sooties evidently carry some of the factors for darkness which appear in greater concentration in sables and black-and-tan as well as in

the blacks derived from these varieties. Sooty, then, appears to be a lower stage of sable in the more complete restriction of non-yellow pigments from the hair.

In addition to the varieties treated above it is necessary to speak of light bellied mice which can not be included in the sable series. I refer to the light-bellied agouti variants reported by Cuénot and Morgan. These variations have been shown to belong to a series of multiple allelomorphs in which the other members are ordinary agouti, yellow, and non-agouti. The light-bellied agouti also arose spontaneously in Little's 1916 crosses between gray-bellied agouti and dilute brown. Such light-bellied agoutis bred true and when crossed with a non-agouti variety they produced in $F_2$ only light-bellied agoutis and normal non-agoutis. This result contrasts strongly with the result of a cross of black-and-tan with wild agouti which produces only sables and gray-bellied agoutis in $F_1$, while in $F_2$ there result yellows, sooties, sables, black-and-tans, agoutis and darkened agoutis. The difference is readily seen to be due to the yellow gamete of the sable series.

To explain the results of the genetic behavior of sables one is led to review the origin of the varieties concerned. The wild house mouse is undoubtedly the ancestral type from which all varieties of fancy mice have descended. Its pelage contains the three fundamental pigments of mice: yellow, black and brown, formed in the mosaic known as the agouti pattern by the presence of a specific gene "A." Each pigment is likewise determined by a gene, Y for yellow, B for black, b for brown (absence of black) and by loss of one or more of these genes, or by the gain of other genes determining the distribution of the pigments present, the whole array of fancy varieties has resulted.

Yellow was shown by Cuénot to be due to a change in the gene "A," resulting in the presence of a restrictive factor which limits the distribution and amount of black and brown pigment, the eyes alone being dark pigmented while black or brown pigments are present in the hair and

skin in such small amounts as to leave the pelage clear yellow. All three fundamental pigments are present in yellow and it is essentially an agouti in which the dark pigments are quantitatively restricted and reduced. This gene acts as the dominant allelomorph of agouti, light-bellied agouti, and non-agouti. Black mice, on the other hand, represent no quantitative reduction in amount of pigment but only the absence of the genes for yellow and agouti. The sables contain a gene allelomorphic to agouti and non-agouti. The evidence shows that this gene is common to yellow, sooty, sable and black-and-tan mice. This gene is a lethal. When it is present in both gametes uniting to form a zygote it causes the death of the zygote. This lethal gene (yellow) might conceivably assume several forms and cause the differences noted among the sable varieties. If such were the case, yellow and sable varieties would be members of a series of multiple allelomorphs of a single gene. Such series have been demonstrated in the gene for white eye in Drosophila by Morgan and his co-workers; in the color gene in the guinea pig by Wright (1915); and in the agouti gene in the mouse itself as quoted previously. But if the black-and-tan mouse or a sooty were due simply to a different form of the yellow gene the difference of their black recessives from ordinary blacks would still remain to be explained. Moreover yellow and the members of the sable series are more closely related to each other than as mere members of a multiple allelomorphic series. They do contain an identical gene, and unlike multiple allelomorphs can be changed one into the other more or less completely. Their difference rather inheres in their possession of modifying genes determining the quantitative increase of black or brown pigments not only in connection with the yellow gene itself but in connection with the genes for black, brown and agouti.

Such modifying genes can not be merely changes in a distributive gene such as agouti or the gene for restriction which causes yellow, for their presence has been demonstrated in non-agouti, non-yellow animals. They

must rather be related to the formative genes for black and brown pigment, so that the number of black and brown pigment granules is increased in proportion to the number of modifying genes present. I have examined microscopically hairs from the mid-dorsal region of a black-sable mouse which was intermediate in color between black-and-tan and yellow, from pure black-and-tan and from clear yellow mice of the same age. The cause of the difference among the hair colors of these three forms was clearly the varying number of black pigment granules. In the yellow hair the dark granules were extremely rare and poorly defined, appearing in many cases as partially mixed with the diffuse yellow ground color. In the sable hair the black granules were more numerous, occurring singly in the distal third of the shaft, while in the proximal two thirds the concentration was greater. Here the granules were large, one granule usually extending across the medullary space. In some cases two granules appeared side by side, and in rare instances I noted rows of three across the hair. In the hairs from the black-and-tan the concentration of granules was three to ten times as great as in the sable hair; the whole shaft was filled with closely packed small black granules. One row was rare; two was common; the rule was three or four rows, while I sometimes found rows of six small granules packed closely into the width of the hair.

If, as I have stated, there exist in mice genes determining the quantitative increase of dark pigments, it should be possible by experiment to test their existence and to determine whether they are Mendelian in behavior or not and whether they are simple or multiple. The data which follows is submitted as a test of the above questions.

According to the provisional hypothesis, black-and-tan being the darkest member of the series should genetically contain the greatest number of modifying genes. The presence of such genes should become apparent if black-and-tan were crossed with a race containing the dark pigments but lacking entirely any of the modifying genes. These conditions were satisfied only by pure wild house

mice, caught at a distance to insure against any contamination from crossing with fancy varieties. These wild mice were regarded as lacking the darkeners; black-and-tan as containing the maximum concentration of darkeners; and after some preliminary experimentation it was decided that six stages in darkness could be distinguished of which the wild was regarded as grade 1 and black-and-tan as grade 6. These did not represent all the grades that actually appeared, for the variation from one to six was practically continuous but such arbitrary points had to be fixed for convenience in observation and record. These grades were standardized by means of type skins for each grade with which each mouse was compared at the age of three weeks and at later intervals throughout life.

The results of crossing black-and-tan with wild agouti are seen in Table I and Fig. 1. The first generation consisted of two classes of young, darkened yellows and darkened agoutis. The mode of the $F_1$ yellows was at grade 3, and their mean grade was 3.3 both practically midway between the parent grades. The mode of the $F_1$ agoutis was at 2 and their mean grade was 2.8 showing that although they represented a blend between the parental types the agouti pattern affords a less favorable background for the development of darkness than does yellow.

Two $F_2$ generations were raised, one by inbreeding the $F_1$ yellows, the other by inbreeding the $F_1$ agoutis. The distribution of $F_2$ yellows shows the increased variability which we have come to expect from such blending characters as size and other quantitative measurements. Evidence of segregation of parental characters appears from the presence of a large number of grade 1 (yellows) and the separation of this class from the other large class (grades 3 and 4) by a small class (grade 2). Segregation of darkness may also be inferred from the large class at grade 5 which contained relatively few individuals in $F_1$. The mean grade of $F_2$ is 3.0 indicating that the average darkness has not changed while the distribution has changed considerably. The same is true in a lesser de-

gree of the $F_1$ agoutis resulting from the inbred $F_1$ yellows and of the progeny of the inbred $F_1$ agoutis. In the latter class strong evidence of the segregation of the undarkened wild is apparent from the large size of grade 1

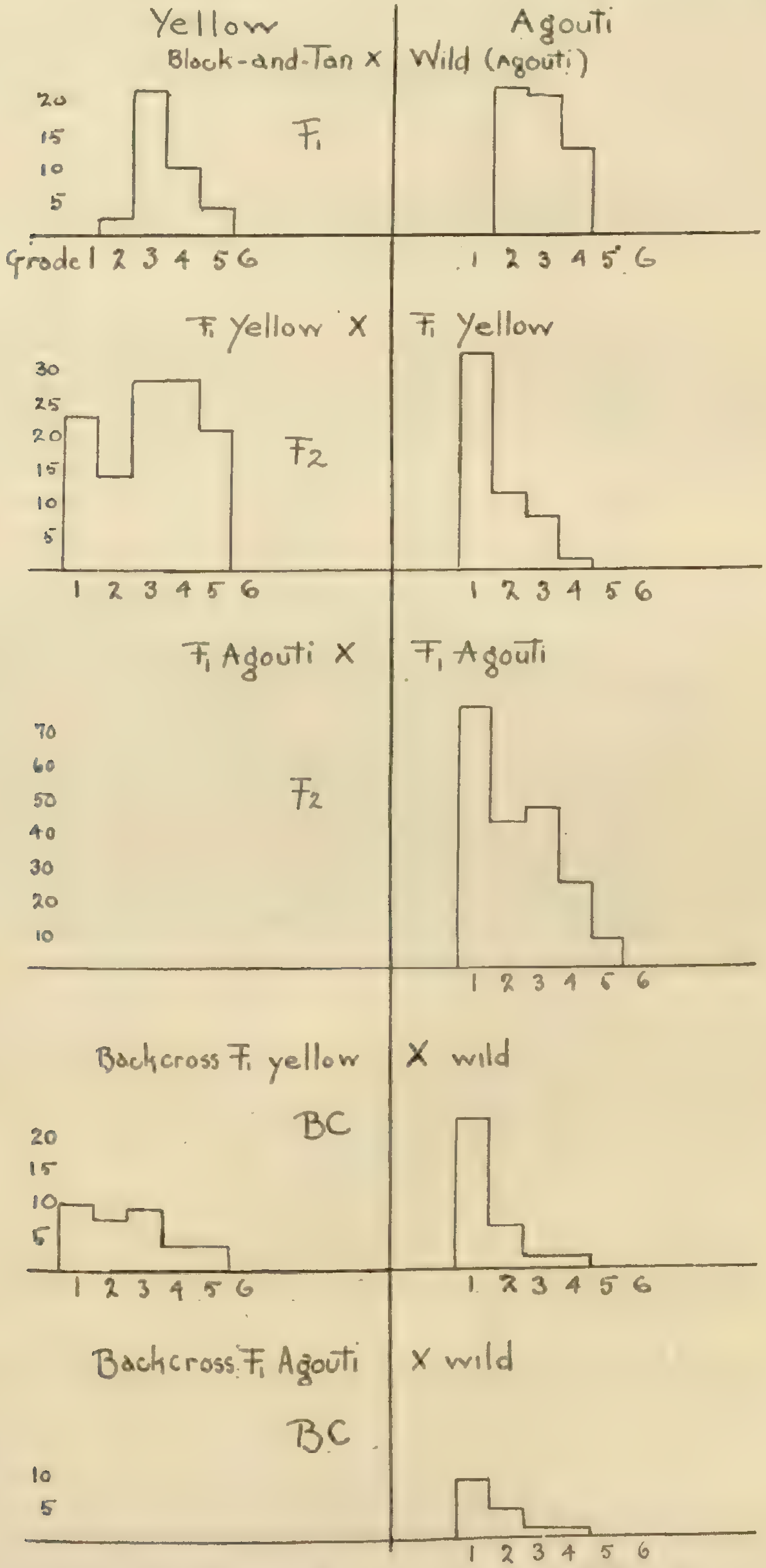


FIG. 1. Crosses of Black-and-Tan with Wild.

TABLE I

CROSSES OF BLACK-AND-TAN WITH WILD AGOUTI

| Cross Number | Parents by Generation and Grade | Yellow Young by Grades * | | | | | | Agouti Young by Grades | | | | | | Total Young | Total Yellow | Yotal Agouti | Total Black | Mean Yellow | Mean Agouti |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 | | | | | | |
| | | Yellow | Sooty | Sable | Dk. Sable | Blk-Tan.Lt. | Blk.-Tan | | | | | | | | | | | | |
| I..... | Blk.-Tan (6) × Wild Agouti (1) | | 2 | 21 | 10 | 4 | | | 23 | 22 | 13 | | | 95 | 37 | 58 | | 3.43 | 2.83 |
| II.... | $F_1$Agouti (2, 3, 4) × Fi Ag. | | | | | | | 78 | 43 | 46 | 25 | 9 | | 211 | | | 57 | | 2.22 |
| III... | $F_1$ Agouti (3) × Wild (1) | | | | | | | 8 | 4 | 1 | 1 | | | 14 | | 14 | | | 1.64 |
| IV ... | $F_1$Sable (3, 4) × $F_1$ Sable (3, 4) | 23 | 14 | 27 | 27 | 21 | | 33 | 12 | 8 | 1 | | | 166 | 112 | 54 | | 3.08 | 1.58 |
| V .... | $F_1$ Sable (3, 4) × Wild | 10 | 8 | 9 | 4 | 4 | | 23 | 6 | 2 | 2 | | | 68 | 35 | 33 | | 2.54 | 1.49 |
| VI ... | $F_2$ Blk. and Tan (light) (5) × Wild | | | 7 | 1 | | | 4 | | | | | | 8 | 8 | 4 | | 3.12 | 1.00 |
| VII .. | $F_2$ Dk. Sable (4) × Wild | 3 | 3 | 6 | 3 | | | 28 | 2 | | | | | 45 | 15 | 30 | | 2.60 | 1.07 |
| VIII.. | $F_2$ Sable (3) × Wild | 3 | 2 | 2 | | | | 8 | | | | | | 15 | 7 | 8 | | 1.86 | 1.00 |
| IX ... | $F_2$ Sooty (2) × Wild | 4 | 3 | | | | | 8 | | | | | | 15 | 7 | 8 | | 1.43 | 1.00 |
| X .... | $F_2$ Yellow (1) × Wild | 8 | | | | | | 10 | | | | | | 18 | 8 | 10 | | 1.00 | 1.00 |

(wild). The average grade of these $F_2$ agoutis is 2.2, slightly lower than the $F_1$ grade while two new grades have been added to the distribution, grade 1 (wild) and grade 5 (very dark agouti). A few back crosses were made of $F_1$ agoutis with wild, and of $F_1$ yellows with wild. These showed likewise segregation of undarkened yellows and undarkened agoutis and lower mean grades than $F_1$—2.5 for yellows and 1.5 for agoutis.

The results thus far had established the presence of a factor or factors for darkness, the formation of intermediates in $F_1$ when crossed with animals lacking it, and the incomplete segregation of parental types in $F_2$. They had not answered our questions regarding Mendelian behavior or number of factors involved. This failure may have been due to incorrect observation, to the failure of the grading scale to distinguish between the types produced or to the actual non-appearance of the types expected. By constant application and regrading it was believed that the error from the first two reasons was low. To consider the third, one must recall what is known of the origin of the black-and-tan variety. It has been for some years a standard breed of the English fancy, built up probably through the constant selection by breeders of the points it now possesses—clear yellow belly and intensely black back with a sheen not duplicated in any other variety or in the hybrids of black-and-tans with other varieties. Since the variety breeds quite true these points must be heritable, and one can hardly expect to extract the pure type of black-and-tan from a cross with wild without practising upon the segregates a selection similar to that which perfected the variety. This involves the supposition that the factors causing the darkness of the black-and-tan are very numerous and extremely small in individual effect.

The $F_2$ darkened agoutis were chosen as the starting point of a selection for darkness which lasted through several generations. These agoutis were easier to grade because they were non-yellow; their litters were larger for the same reason, and it was also desired to know

whether the fact that black-and-tans were always heterozygous for black was a cause of their darkness. The re-

TABLE II

CROSSES OF DARK AGOUTIS INTER-SE

| Parents | Grade Distribution of Young | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 5.5 | 6 | Total Ag. | Mean Grade | Blk. |
| XI. 1 × 1 | 32 | | | | | | | 33 | 1.00 | 1 |
| XII. 2 × 2 | 11 | 21 | | | | | | 33 | 1.60 | 1 |
| XIII. 4 × 2 | 11 | 18 | 6 | 3 | | | | 38 | 2.02 | 2 |
| XIV. 4 × 3 | | | 6 | 5 | | | | 11 | 3.45 | 1 |
| XV. 4 × 4 | | | 7 | 6 | | | | 13 | 3.46 | 0 |
| XVI. 5 × 4 | | 1 | 10 | 8 | 16 | 5 | 4 | 51 | 3.83 | 13 |
| XVII. 5 × 5 | | 3 | 17 | 33 | 28 | 18 | 6 | 105 | 4.38 | 18 |
| XVIII. 6 × 4 | | 1 | 5 | 19 | 19 | 8 | 13 | 68 | 4.66 | 2 |
| XIX. 5.5 × 5.5 | | | | 4 | 4 | 8 | 5 | 21 | 5.23 | 7 |
| XX. 6 × 5 | | | 6 | 20 | 41 | 47 | 21 | 135 | 5.09 | 30 |
| XXI. 6 × 6 | | | | 6 | 16 | 23 | 27 | 72 | 5.45 | 18 |
| XXII. 6 × 1 (wild) | 13 | 26 | 19 | 8 | 6 | | | 72 | 2.55 | 0 |
| I. Blk.-tan × wild | | 23 | 22 | 13 | | | | 58 | 2.83 | 0 |

sults of mating together dark agoutis of various grades is shown in Table II and Fig. 2.

The table shows plainly that the variation in darkness is practically continuous. Animals of grade 1 proved to be pure wild segregates entirely lacking the darkeners. Grades 2, 3 and 4 contained the darkener but never produced by recombination any animals darker than their own grade. Grades 5, 5.5 and 6 produced grades both darker and lighter than their own, proving them to be heterozygous in the modifiers. None of these darkest grades proved to be pure dark segregates. Even grade 6 which was entirely black with a gray belly and quite comparable in darkness to black-and-tan produced animals of grade 1 (light segregates) when crossed with wild agouti. These grade 1 agoutis were tested and found to lack any modifiers for darkness. Therefore grade 6 in darkness was not homozygous as regards the darkening modifiers.

The supposition that the darkness of the dark agoutis might be due simply to their being heterozygous for black was disposed of by the results of the dark agouti crosses, for of eighteen $F_2$ dark agoutis thoroughly tested, twelve were heterozygous for black and six were homozygous

agoutis. In subsequent generations selection was in the direction of darkness. That this was not accompanied by selection of heterozygotes is shown by the figures for dark agoutis of $F_3$, $F_4$ and $F_5$ which were tested. In generations $F_3$ and $F_4$ there were twenty agoutis heterozygous for black to 8 homozygous agoutis; in $F_5$ four heterozygotes to three homozygotes, and the homozygotes com-

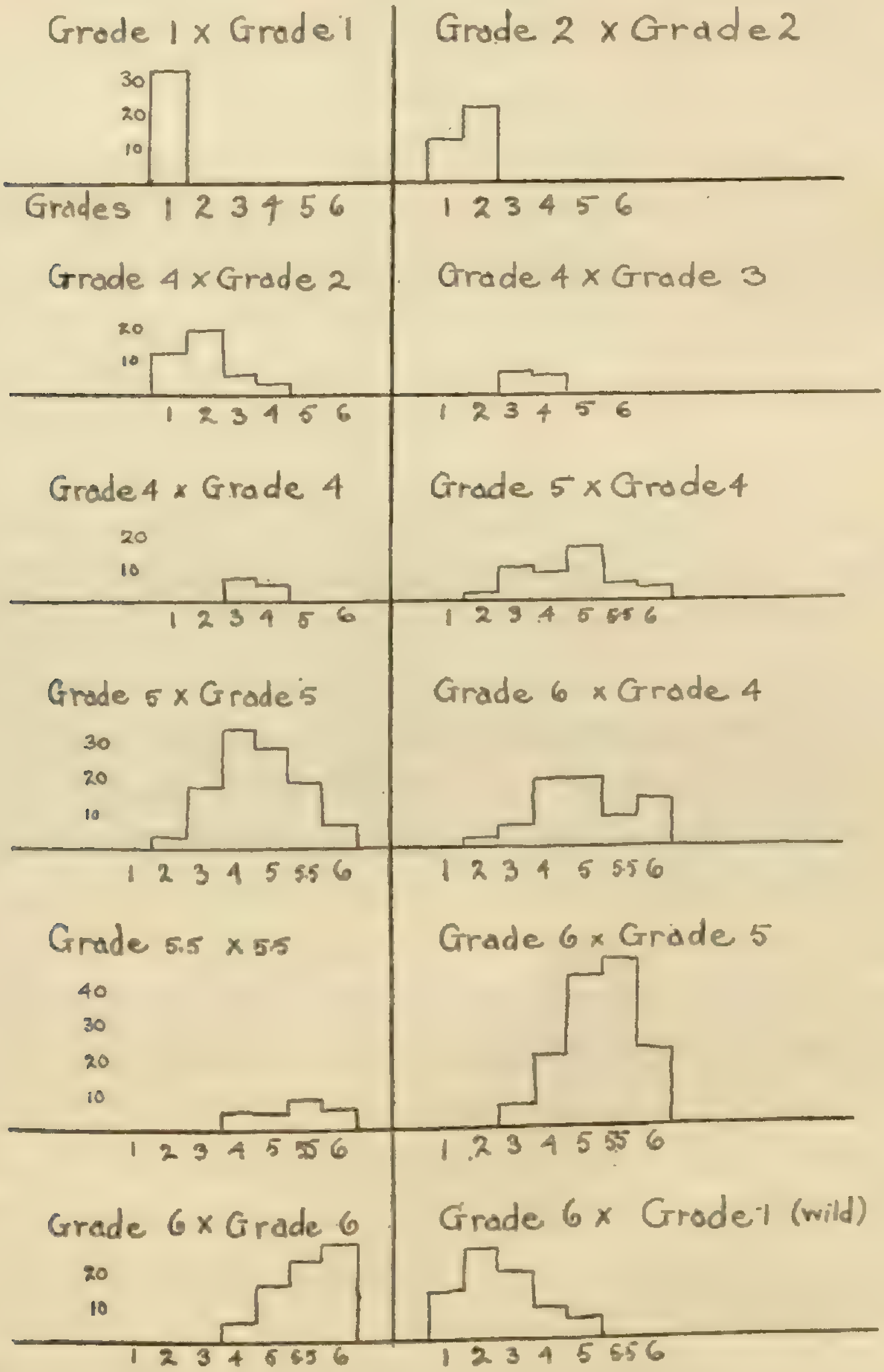


FIG. 2. Crosses of Dark Agouti Inter-Se.

prised some of the darkest mice produced. Another explanation for the darkness of the agoutis must be invoked and the data indicate that the only alternative is the ac-

quisition by the dark agoutis of the peculiar and puzzling darkness from the black-and-tan race.[1]

Up to his point the data has involved crosses of darkness with its absence—and crosses of dark animals of various grades inter se. Multiple factors appear to be involved and yet no evidence of Mendelian behavior has been adduced except the segregation of lack of darkness without accompanying segregation of darkness.

Many additional crosses have been made in which the darkeners from the sable series have been transferred into other varieties with results similar to those outlined above. The most extensive of these secondary experiments involved crosses of black-and-tan with brown (chocolate) mice to test the black-and-tan (yellow) gamete and to separate if possible the darkening modifiers (see Table III and Fig. 3). This cross brought about the union in the first generation of gametes with full darkness (black-and-tan) with a uniform set of gametes from the brown race all of which supposedly lacked the darkener. The first generation from this cross showed, as was expected, the dominance of black, all young being black pigmented. Approximately one half were self black which on being tested proved to be heterozygous for brown. The other half were a lightened black-and-tan (Table III, cross 23) with some variation but none were as black as pure black-and-tan. The number of dark pigment granules in the dorsal hairs was reduced and the yellow pigment substratum was thereby allowed to show at bases and tips of hairs especially in the older animals. From these light black-and-tans was bred an $F_2$ generation (cross 26) consisting of mice with black pigment and

[1] Morgan in 1914 reported the appearance in his experiments of mice to which he gave the name "new gray," which were noted first in the offspring of a pair of cinnamon mice. They "looked like chocolates, but . . . showed on later inspection distinctly ticked hair. One of these new grays bred to black (heterozygous) gave some chocolates, black, new grays, and one very dark, almost black, mouse." The above descriptions apply quite accurately to cinnamon and agouti mice which I have raised from crosses with one of the sable series and I have little doubt that the darkness of Morgan's new grays was derived originally from some mouse carrying the "darkener."

mice with brown pigment in approximately the ratio 3:1. Of the blacks about one third were self black; the others were yellows, sooties and black sables with varying amounts of black in the fur, much like the $F_2$ array in the

TABLE III

CROSSES OF BLACK-AND-TAN WITH BROWN

| Cross No. | Parents | Yellow and Black Young | | | | | | | | | | Yellow and Brown Young | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 5.5 | 6 | Mean | Blk. | Tot. | 1 | 2 | 3 | 4 | 5 | 6 | Mean | Brown | Tot. |
| 23 | Blk.-tan × Br. . . . . | | | | 4 | 17 | 6 | | 4.96 | 19 | 46 | | | | | | | | | |
| 24 | $F_1$BT × $F_1$BT . . . . . | 2 | 1 | 2 | 3 | 12 | 26 | 14 | 5.15 | 26 | 86 | 0 | 2 | 1 | 1 | 8 | 10 | 5.05 | 12 | 34 |
| 25 | $F_1$BT × brown . . . . | 5 | 3 | 11 | 2 | 4 | 2 | | 3.00 | 30 | 57 | 9 | 2 | 7 | 0 | 10 | | 3.00 | 26 | 54 |
| 26 | BT × Br. and tan 6 | | | | 1 | 1 | 0 | 2 | | 3 | 7 | | | | | | | | | |
| 27 | $F_1$BT × $F_1$BT from cross 26 . . . . . . . | | | | 1 | 2 | 5 | 17 | 5.74 | 7 | 32 | | | | | 3 | 3 | | 5 | 11 |

black-and-tan × wild agouti cross. In this $F_2$ distribution were seen, besides all grades of intermediates, segregates of both sorts, viz., yellows of grade 1 and black-and-tans of grade 6, although the latter did not retain their darkness throughout life and when bred proved not to be pure segregates. The $F_2$ mice with brown pigment were similarly divided into yellows and brown sables with varying amounts of brown in the fur and self brown in the ratio 2:1. Of the yellow-browns some were evidently counterparts of black-and-tan with the black pigment replaced by brown. To these the name brown-and-tan was given; the other yellow-browns paralleled the yellow-black series although the lower and intermediate grades were less well represented, due perhaps to the difficulty of distinguishing between yellows with considerable brown pigment and yellows with smaller amounts of black pigment. Breeding tests were used in doubtful cases.

The $F_1$ light black-and-tans, when backcrossed to browns (Table III, cross 24), gave equal numbers of all four sorts, viz., yellow-blacks, self blacks, yellow-browns and self browns. The mode of these yellow-blacks was in the middle class (grade 3) in contrast with the mode at 5.5 in the straight $F_2$.

These crosses of black-and-tan with brown show the independence of the darkeners from black pigment for in these experiments the modifiers have been detached from

the black pigment on which they operate in the pure black-and-tan and transferred to brown pigmented yellows where their action is similar. Their independence of yellow was illustrated by their action on agouti mice.

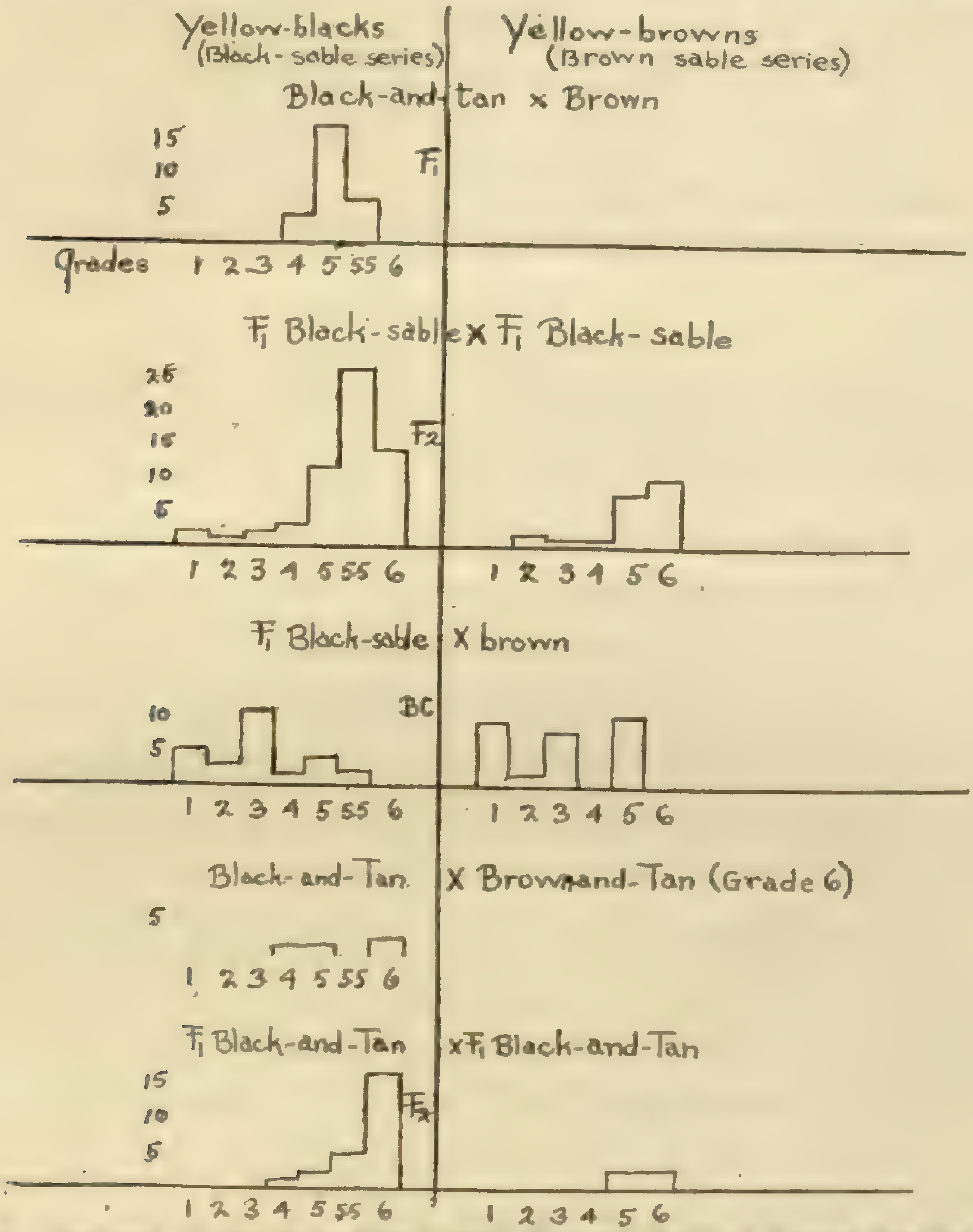


FIG. 3. Crosses of Black-and-Tan with Brown, Yellow Young by Grades.

The intermediate nature of $F_1$ in the black-and-tan × brown cross and subsequent segregation of dark and undarkened forms is not as clear as in the agouti crosses, for it is probable that the brown parent contributed factors comparable to the darkeners which have acted to make both $F_1$ and $F_2$ darker than if the darkening factors had come only from the black-and-tan parent. The browns used were derived from a yellow variety known as red. Both the reds and their brown recessives are more intensely colored than any other yellows or browns, and in fact have given evidence in later experiments of differing from ordinary varieties by intensity factors similar to the darkening factors of black-and-tan.

The results throughout indicate that we are dealing here with genetic factors similar to those which have produced such quantitative differences as size in the races of rabbits studied by Punnett. The results obtained from crossing large and small races of rabbits agree in the production in $F_1$ of animals genetically intermediate between the parent races, and in the evidence of subsequent segregation of the smaller size without segregation of the larger. The same phenomenon appears in the present study for light segregates have appeared but never the dark. Placing the causes of darkness in mice in the same category with the causes of size differences, which have not yet been made clear, is an admission of the unsuitableness of the material rather than of the insoluble nature of the problem. Either the best material for the investigation of such grade variations has not yet been found or else the technique of observation and measurement of such genetic differences as distinguished from non-heritable differences has not yet been evolved. The correct interpretation of such differences must await an investigation combining an optimum of material and method.

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# SHORTER ARTICLES AND DISCUSSION

## HYBRIDIZATION AND EVOLUTION

Some years ago the writer made a cross between the two species *Nicotiana rustica* L. and *Nicotiana paniculata* L.[1] Since the hybrids obtained through this mating are not completely sterile, some biologists may perhaps maintain they are not distinct species, but such a claim is wholly arbitrary. In a sense, a species is a human concept and as such its definition may be carried to any ridiculous extreme, yet there is no more striking biological fact than that *in general* the great groups of living things do fall into specific subdivisions which many criteria show to be distinct, discontinuous, without intermediates. In two such groups fall the above types. Though their ranges overlap, they differ from each other in leaf, stem, flower and habit of growth much more than do several other pairs of species within the same genus between which hybridization is impossible, or where the hybrid is sterile.

The cross between these two species gives an $F_1$ generation intermediate between the two parents, and as uniform in each character as either parental group.

Few of the male or the female gametes are viable, yet by careful attention to pollination, from one to twenty seeds can be obtained in the capsules, where normally two hundred to three hundred seeds are found. These seeds produce an $F_2$ generation which is inordinately variable. No two plants are similar, and numerous types can be picked out which if found in the wild would undoubtedly be classed as different species. In genetic terms, the behavior of the two species may be described as follows: They differ in an extremely large number of inherited factors; and owing to these numerous differences, many of the otherwise possible combinations of $F_1$ gametes, are not functional. A huge percentage of expected combinations of both gametes and zygotes are thus eliminated.

The factors which in combination produce normal fertility,

[1] A detailed account of the genetic facts found in this study, has not yet appeared. A preliminary paper was published in the *Proc. Amer. Phil. Soc.*, 54: 70–72, 1915.

recombine in the Mendelian sense, quite as do the factors controlling the form of leaf and flower. The result is that after a few generations of selection one may obtain a variety of strains, uniform within each line, so fertile as to yield capsules with over ninety per cent. of the normal quota of seed, and so different from one another that the extreme types are more unlike than the two original species used in the cross.

After three years of selection ($F_5$), eight such strains remained out of a large series of selections studied earlier. It seems hardly necessary to describe the differences they exhibited. Suffice it to say that the smallest type was about 20 cm. in height with small smooth oval leaves, and the largest was nearly 200 cm. in height with wrinkled cordate leaves some of which were 50 cm. in length.

*These eight strains were crossed in all possible combinations, and every $F_1$ generation exhibited as high a degree of fertility as that shown by the parents.*

To the writer it seems possible that these results have a bearing on certain theoretical problems which may not be clear at first sight.

A few years ago Lotsy[2] published an extended paper based on a very limited number of crosses in the genera *Nicotiana*, *Pisum*, *Petunia* and *Antirrhinum*, where partially sterile $F_1$ plants produced exceedingly variable progeny,—results wholly comparable with our own. From these observations, neglecting all evidence of the appearance of mutations in controlled pure lines, Lotsy founded a theory of evolution. His arguments were based upon five assumptions: (1) that all characters obey the Mendelian law of heredity, (2) that acquired characters are never transmitted, (3) that homozygotes are absolutely constant in succeeding generations, (4) that there has been no proof of variation independent of crossing, and (5) that the variations observed after crossing are sufficient to account for evolution.

Naturally numerous criticisms can be made against this extreme interpretation. One need only inquire as to the source of the original variations which are to form the basis of all Mendelian recombinations, to show the untenability of the position. On the other hand, it will be admitted by all that hybridization has played some part in evolution, and it is of some importance to endeavor to determine the limits of its rôle.

[2] Lotsy, J. P., "La théorie du croisement," *Arch. Néerland. Sci. Exact. et Nat.*, III, B, 2: 1–61, 1914.

The observations of the writer on the enormous variability of the $F_2$ generations arising from partially sterile $F_1$ generations produced by crossing species, led him to suspect that such combinations might be the basis of a great deal of variability responsible for evolution under domestication. A careful survey of the evidence relating to the origin of modern horses, cattle, sheep, swine, dogs, guinea pigs, fowls, ducks, and geese on the one hand, and varieties of wheat, corn, barley, oats, rye, apples, grapes, roses and begonias on the other hand, shows that in every case several related wild or semi-wild species exist which will cross together and yield partially fertile offspring. Doubtless many other species which have shown great improvement under domestication, would be found to have wild relatives which behave similarly, should they be investigated. Both the historical and the experimental evidence, therefore, point to hybridization, and particularly to species of hybridization, as the great single cause of evolution under domestication.

At the same time, one must not confuse evolution under domestication with natural evolution. The outstanding biological feature characteristic of the varied groups of domestic animals and of cultivated plants, is the perfect fertility within each group. A marked peculiarity of the great majority of natural species is their sterility with one another, the origin of which has long been a stumbling block to writers on evolutionary biology. Our own experimental evidence, as far as it goes, and observations on domestic forms which presumably have originated from combinations of two or more wild species, yield not the slightest indication of a tendency toward the production of segregates that exhibit either incompatibility in crosses or sterility of the individuals produced by hybridization.

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## THE MEASUREMENT OF LINKAGE

LINKAGE is a name for that tendency sometimes shown by genes to maintain in hereditary transmission their previous relations to each other. Thus if two linked genes, A and B, enter a cross together, in the same gamete, they will oftener than not be found together in the gametes formed by the cross-bred individual. And if the same two genes enter the cross separately,

one in the egg, the other in the sperm, then oftener than not they will be found apart, in different gametes formed by the cross-bred individual.

Where no linkage exists between two genes, A and B, it will be wholly a matter of chance whether they go together or not, no matter what their previous relation was. We say that they "assort independently," as genes do in ordinary Mendelian inheritance, such as was known to Mendel. In such cases change of relation occurs in the long run in half (or 50 per cent.) of all cases. Such change of relation is called "crossing over." Linkage evidently will be shown by a falling below 50 in the percentage of cross-overs. The more cross-overs decline below 50 per cent., the stronger will be the linkage indicated, until when no cross-overs occur, we call the linkage complete or perfect. Accordingly 0 and 50 per cent. will be the limiting values for cross-overs indicating linkage. But it is conceivable that cross-overs might occur in excess of 50 per cent. What would their significance be? Not linkage, not a tendency to maintain relations previously existing between genes, but a tendency to *change* those relations, to go apart when previously together, and to get together when previously apart. We are not acquainted with any such tendencies as these, and it is difficult to imagine how they might arise, but it is certain that they would be the opposite of linkage and would need a different name, if observed.

It is evident that the strength of linkage increases, as the cross-over percentage decreases below 50. As a measure of the strength of linkage, we might then take the difference between 50 and the observed cross-over percentage, as I have elsewhere suggested (Castle, 1919). This would give us a numerical grade of linkage strength on a scale of 50. But since we are more accustomed to grading on a scale of 100, it will perhaps be better to double values thus obtained. Our grading scale of linkage strengths will then run thus:

| Cross-over Percentage | Linkage Strength |
|---|---|
| 50 | 0 |
| 40 | 20 |
| 30 | 40 |
| 20 | 60 |
| 10 | 80 |
| 0 | 100 |

By this method we can compare the linkage strength between

any two pairs of genes without stopping to reverse the relations indicated by cross-over percentages. For example the following linkage relations are shown by the genes of rats and mice (Dunn).

| Genes | Cross-over Percentage | Linkage Strength |
|---|---|---|
| Albinism—red-eye, rats .............. | 1.8(?) | 96.4 |
| Red-eye—pink-eye, rats .............. | 18.3 | 63.4 |
| Albinism—pink-eye, rats ............. | 21.1(?) | 57.8 |
| Albinism—pink-eye, mice ............ | 14.6 | 70.8 |

The strongest linkage here indicated is that between albinism and red-eye in rats, next comes that between albinism and pink-eye in mice. But albinism and pink-eye in rats show less linkage than in mice. The three genes, albinism (*c*), red-eye (*r*) and pink-eye (*p*) in rats are apparently arranged in linear fashion thus:

c......r....................p
0 2(?) 21

This kind of a diagram is what Morgan, Bridges and Sturtevant (1919) have made familiar to us under the name of "chromosome map." Not to prejudice the case for or against the chromosomes, we might perhaps call it a linkage map or map of a linkage system. In its construction we use cross-over percentages as direct measures of map distances, but in *Drosophila* at least only distances relatively short have been found to be strictly comparable. Beyond distances of about 5 units (cross-over percentages) it is found that double or triple cross-overs become increasingly common and thus decrease the apparent number of breaks in the linkage chain between two genes. So that long map distances are based, not on directly observed cross-over percentages between the more distant genes, but on summation of intervening short distances, it being assumed that the arrangement is in all cases linear. While this latter assumption is not to be accepted for all cases without proof, it must be admitted that for *Drosophila* at least the evidence for a linear arrangement is very strong and no insuperable objections can be raised against it.

Map-distances have been found in the "first chromosome" linkage group of *Drosophila* exceeding 60, and in the "second chromosome" group exceeding 100. But in no case does the observed cross-over percentage between two genes, however remote, of the same linkage group exceed 50. This means that

beyond very short distances cross-over percentages do not increase in proportion to distance. The linkage group forms a means of holding genes together, however distant they may be from each other, so that, as one goes, all have a tendency to go. The linkage map will give us a diagrammatic view of the relations to each other of the genes composing a linkage system. It is based on the *shorter observed cross-over percentages*, or where longer distances are used, they must be first corrected for double and triple crossing-over. See in this connection the valuable Table II. of Haldane (1919) which provides a ready means of converting map distances into cross-over percentages or *vice versa*, and so of predicting undetermined linkage relations. It is based on a mathematical examination of the linkage system of the first chromosome of *Drosophila*. A table of linkage strengths will show us, without reference to distances involved, to what extent the movements in gametogenesis of one gene are correlated with those of any other gene. It is based on the *unmodified cross-over percentages observed*, whether the map distances involved are great or small. Linkage strengths can never exceed 50 on a scale of 50, 100 on a scale of 100, whereas map-distances may be extended indefinitely with the discovery of new genes.

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## IS THERE LINKAGE BETWEEN THE GENES FOR YELLOW AND FOR BLACK IN MICE?

In a recent number of this journal Dunn[1] has given data showing a deficiency of black young in a family of yellow mice.

[1] Am. Nat., 53: 558–560, 1919.

Thus in a cross of two yellows, one of them probably heterozygous for both black and brown and the other for brown only, the offspring totalled fourteen yellow and four brown, the expectation being twelve yellow, three black and three brown. The yellow descendents of this mating when bred to browns are expected to give two yellows (one heterozygous for both black and brown and one heterozygous for brown only) to one black to one brown. The actual numbers obtained were eighteen yellow, one black, and ten brown, and the expected numbers fourteen yellow, seven black and seven brown. Dunn has summarized these data according to the percentage of young of each sort produced, as follows:

| | Yellow | Black | Brown |
|---|---|---|---|
| Per cent. expected | 66.6 | 16.6 | 16.6 |
| Per cent. observed | 62.0 | 3.4 | 34.5 |

In the total number of young observed, the chances are equal that the 16.6 per cent. expected black young might go as high as 30.8 per cent. or as low as 2.4 per cent. It is therefore apparent that neither the 3.4 per cent. black nor the 34.5 per cent. brown are significantly outside the limit of probable variation due to chance.

Although not including chance fluctuation as one of the three theories capable of explaining his observed facts, Dunn evidently feels the need for larger numbers of young before considering random sampling eliminated.

It is interesting however to see just what evidence there is in Dunn's data that black and yellow are linked. Apparently the only facts in support of this hypothesis is the deficiency of blacks and the slight excess of browns referred to. Significant evidence for the expected excess of yellows carrying both black and brown as compared with those carrying brown only is not obtained. Of seventeen such yellows tested, ten carried both black and brown and seven brown only—exact equality or 8.5 of each is the Mendelian expectancy. The excess of yellows carrying black and brown is 8.8 per cent. as against non-yellow browns of 17.9 per cent. The deficiency of yellow carrying brown only is 8.8 per cent. as against a deficiency of blacks of 13.3 per cent. The sum of the departures from the expected equality in *yellows* is 17.6 per cent., while in *non-yellows* it is 31.2 per cent., or almost twice as much. The discrepancies in the yellow indi-

viduals are even more within the possibility of chance fluctuation than those of the non-yellows.

Dunn states that linkage between Y and B "affords a satisfactory explanation of the observed facts in harmony with other cases of linkage." However, one of the essential points of linkage is that members of a multiple allelomorph series are linked to a given gene in the same degree. Cuénot,[2] Morgan,[3] Sturtevant,[4] and the writer,[5] have shown that the genes for yellow and for agouti in mice are allelomorphic. Many investigators, including Durham,[6] Detlefsen,[7] and the writer,[8, 9] have shown that agouti and black are not linked in inheritance—yet according to our present knowledge of linkage all genes in the same locus are equally linked with any other given gene, and these crosses should show linkage between agouti and black to a degree equal to that of linkage between black and yellow. Dunn has not emphasized this point sufficiently.

Furthermore, if there is any significance other than random sampling in the peculiar ratios reported, there is another possible explanation, not considered by Dunn, which avoids hypothecating linkage between yellow and black. If a lethal factor was closely linked with black in the particular family under consideration, and if this lethal was effective in a heterozygous condition in non-yellow mice—but not in yellow mice, the observed results would be explicable as follows:

| | |
|---|---|
| Let Y equal yellow, | y equal non-yellow. |
| B equal black, | b equal brown. |
| L equal lethal, | l equal normal. |

Yy $\widehat{BL}$ $\widehat{bl}$ equals yellow heterozygous for black and lethal.
Forms gametes:

| | | | |
|---|---|---|---|
| Y$\widehat{BL}$<br>y$\widehat{BL}$<br>Y$\widehat{bl}$<br>y$\widehat{bl}$ | commonly | Y$\widehat{bL}$<br>y$\widehat{bL}$<br>Y$\widehat{Bl}$<br>y$\widehat{Bl}$ | rarely |

[2] *Archiv Zool. Exp. et Gen.* (4), Vol. 8, 1911.
[3] AM. NAT., 48, pp. 449–458, 1914.
[4] AM. NAT., 46, pp. 368–371, 1912.
[5] *Sci.*, N. S., 38, p. 205, 1913.
[6] *Jour. Genet.*, I, pp. 159–178, 1911.
[7] *Genetics*, 3, pp. 573–598, 1918.
[8] Carn. Inst. of Wash., No. 179, 1913.
[9] AM. NAT., 47, pp. 760–762, 1913.

Crossed with brown normal yy$\breve{b}\breve{l}\breve{b}\breve{l}$ such a yellow would give the following zygotes:

| | | |
|---|---|---|
| Yy $\widehat{BL}$ $\widehat{bl}$ | Yellow heterozygous for black and lethal | commonly. |
| yy $\widehat{BL}$ $\widehat{bl}$ | *Black lethal; dies** | |
| Yy $\widehat{bl}$ $\widehat{bl}$ | Yellow carrying brown normal | |
| yy $\widehat{bl}$ $\widehat{bl}$ | Brown normal | |
| Yy $\widehat{bL}$ $\widehat{bl}$ | Yellow carrying brown and lethal | rarely. |
| yy $\widehat{bL}$ $\widehat{bl}$ | Brown lethal; dies* | |
| Yy $\widehat{Bl}$ $\widehat{bl}$ | Yellow normal heterozygous for black | |
| yy $\widehat{Bl}$ $\widehat{bl}$ | Black normal | |

The death of the rare brown lethal individual would not be noticed, for the common death of black lethals would leave a distinct excess of brown normals.

This hypothesis is capable of experimental test and involves a lethal mutation in an entirely new factor which presupposes no generality of the process in all yellows and agoutis; and simply assumes that yellow, when present, hampers the action of the lethal in much the same sort of way that it hampers the activity of the black forming factor in the skin and hair.

The above hypothesis is advanced simply as an additional possibility for test in case something more than chance fluctuation due to random sampling is involved.

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## CREPIS—A PROMISING GENUS FOR GENETIC INVESTIGATIONS

To all who are familiar with the recent advances in our knowledge of heredity, which were made possible largely through the investigations of Morgan and others with the fly, *Drosophila melanogaster*, especially to those who have followed the development of the chromosome theory of heredity with its correlative theories of mutation and evolution, the urgent need of extensive corroborative evidence from other animals and plants must be forcibly clear. Although it appears inconceivable that the conclusions reached from the drosophila investigations are not applicable in all their essential features to all animals and plants,

yet it can not be denied that many biologists are not yet committed to the acceptance of these conclusions as of general application. It is obvious that extensive corroborative evidence, derived from other genera of animals and plants, would be of paramount value in firmly establishing these far-reaching conclusions. It, therefore, becomes one, who allies himself with those biologists who believe in the present importance and future promise of this collection of genetic evidence, derived as it is, almost entirely from a single species of insects, to consider most carefully the selection of other material with which to test the various hypotheses that have been proposed in order to interpret the great mass of drosophila data consistently.

It is encouraging to note the energetic efforts of a number of investigators to obtain a corresponding collection of data from other species of *Drosophila.* As yet, however, little more than a beginning has been made, particularly with the genetic investigations on these species, because it is necessary first to find the comparatively rare mutant individuals with which to experiment. No other genus of animals thus far reported upon possesses so many features favorable to genetic study as does *Drosophila,* although it is probable that other of the lower animal groups will in time furnish material just as valuable. In plants, the only species in which genetic analysis has proceeded far enough to establish the identity of a considerable number of hereditary factors or genes, are the garden pea, sweet pea, snapdragon, maize, barley and wheat. In most of these and in some other plants evidence of linkage of characters in inheritance has been obtained, but in none has the number of linked groups been shown to correspond with the number of chromosomes in the germ cell and because of the relatively large number of chromosomes in these species it will probably be some time before any considerable body of corroborative evidence can be accumulated from them.

In addition to a low chromosome number there are several other desiderata which the ideal form for genetic investigations should possess. It must display numerous germinal variations. It must be prolific and easily reared. It should have a short life cycle so as to permit of the maximum number of generations within a given time. Furthermore, in the case of a plant, it should be self-fertile, so as to permit of establishing pure lines; it should be easily hybridized; and it should flourish when grown under glass.

A brief life cycle is extremely important because numerous generations must be raised in order to secure adequate data for the analysis of more complicated genetic problems. In this respect, no sexually propagated flowering plants can compare with the insects. On the other hand, certain highly desirable features possessed by plants are either impossible or very difficult of realization in animals. For example, asexual reproduction can often be resorted to in plants when it is desired to perpetuate a particular individual for comparison with later generations. But the most important point of superiority of plants over insects for genetic study is the greater possibility in plants of securing hybrids between different species. That this advantage should receive considerable weight will be admitted by all who recognize the need of studying hybrids between species having different chromosome numbers. The desirability of such investigations has been mentioned recently by Morgan (1919) as follows:

The theory that the chromosomes are made up of independent self-perpetuating elements or genes that compose the entire hereditary complex of the race, and the implication contained in the theory that similar species have an immense number of genes in common, makes the numerical relation of the chromosomes in such species of unusual interest. This subject is one that could best be studied by intercrossing similar species with different numbers of chromosomes, but since *this would yield significant results only in groups where the contents of the chromosomes involved were sufficiently known to follow their histories,* and since as yet no such hybridizations have been made, we can only fall back on the suggestive results that cytologists have already obtained along these lines.

I have italicized one clause in the above paragraph in order to emphasize the importance of extensive genetic analysis in those particular species which are to be used in intercrossing experiments. It is not sufficient that the species have low numbers and different numbers; it is also necessary that the inheritance of a sufficient number of characters in each species be studied so as to establish the linked groups of characters or genes corresponding to the chromosomes of each species. Only then can the contents of the chromosomes involved be sufficiently known to follow their histories in the hybrids.

Thus we find several excellent reasons for seeking among plant materials for a group of species which possess as many as pos-

sible of those features most favorable to securing the desired results.

With this explanatory introduction let us consider briefly the present state of our knowledge of *Crepis* with reference especially to its promise of usefulness in genetic studies.[1] This genus belonging to the chicory tribe of the Compositæ contains about 200 species (according to Index Kewensis) which are widely scattered, the genus being represented by indigenous species in every continent and in Australasia. Just how great is the diversity in morphological characters within the genus remains to be seen, but the wide distribution of the group as a whole and of some of the individual species would lead one to expect a large number of diverse characters and many different combinations of the same. The descriptive connotation of many of the specific names also indicates a remarkable diversity among these forms. For example, there are giants and pigmies, there are forms with bristly, woolly, floury, and glandular pubescence as well as glabrous forms, there are four or more flower colors and one species is named "bicolor." This expectation has been borne out by such observations on preserved and living specimens as the writer has been able to make. There are annual, biennial and perennial species which should prove to be very interesting forms for interspecific hybridization studies. Finally, within at least two of the individual species, there certainly exists a remarkable diversity of forms.

But it is not for its wealth of variation alone that this genus is especially interesting to geneticists. The cytological investigations which have been made on a dozen or more species of *Crepis* reveal a most interesting situation as regards chromosome numbers. There is at least one species (possibly two or three) having only 3 for the haploid number of chromosomes, a group of six or seven species with 4 chromosomes, another group of four species with 5, a single species with 8, another with 9, and still another with 20 chromosomes as the reduced number. The absence of a common denominator greater than one for this series of numbers has caused some interesting speculations as to the method of derivation of one species from another (Rosenberg, 1918). Several cytologists have also noted the fact that the chro-

[1] This paper is a preliminary communication offered mainly for the purpose of calling attention to this promising material. A few species have been under investigation at the University of California for about three years and will be discussed more fully in a future publication.

mosomes themselves in these species are unusually favorable objects of study, one of my correspondents going so far as to predict that in time *Crepis* will become as famous and useful for laboratory work as *Ascaris* is to-day. But the important consideration in the present discussion is the fact that we have here several species with the same chromosome number as *Drosophila melanogaster* and at least one species with one less chromosome pair. Obviously, if some of these species with the smallest chromosome numbers are highly variable, existing in a large number of distinct varieties or forms, they should serve as excellent material for genetic study especially if they possess the other advantageous features already mentioned.

For at least two such species I can report very great promise as objects of genetic research. *Crepis capillaris* (*virens*)[2] with three chromosome pairs (Rosenberg, 1909, 1918; Digby, 1914) and *C. tectorum*[3] with four pairs (Juel, 1905; Rosenberg, 1909, 1918) both exhibit polymorphism to a remarkable degree. This is evidenced by the diversity of forms referred to these species in the herbaria of the Royal Botanic Gardens at Kew and of the Museum of Natural History in Paris. In both species it seems to be merely a matter of sufficiently extensive seed collection that is required in order to secure a sufficient number of allelomorphic pairs of characters to make possible the desired genetic analysis. My cultures of *C. virens,* which have been grown from seed secured from various foreign countries as well as in California, have already yielded several pairs of contrasted characters which will soon furnish a nucleus of genetic data on this species.

These two species are also very prolific, considering the plant as a whole, there being several or many heads on a plant and each head bearing 5 to 15 fertile achenes in *virens* and 30 to 40 in *tectorum.* Unfortunately an individual flower produces but a single seed and the flowers are so small as to make the work of hybridization rather tedious when absolute control is exercised through castration of the unopened flower. But, while this method is essential in original crosses, it usually is not necessary to castrate many flowers for any one cross, and when it comes to

[2] The nomenclature of this species is somewhat in doubt. Both Robinson and Fernald (1908) and Britton and Brown (1918) name it *C. capillaris* (L.) Wallr., but certain European botanists seem to have retained the name *C. virens* L. for this species.

[3] *C. tectorum* L.

making back crosses on a large scale, it may be practicable to depollinate the flowers of the intended female parent with a water jet instead of actually castrating the buds.

As regards other desiderata to be considered in selecting material for genetic study these two species are very promising. They are easily reared in greenhouse or field, the seeds germinating quickly in glass germinators, thus permitting easy manipulation and careful checking of viability when desired. The life cycle varies from three to six months except in rare cases of retarded development and little or no rest period is necessary in the seed stage, so that it is possible to grow two or three generations in a year with proper facilities for culture under glass. Partial or complete self-fertility is the rule in both these species, although in some strains of *virens* the individual plant is nearly self-sterile. No evidence of parthenogenesis or apogamy has been found in these species. In general, therefore, it will be possible to secure numerous sexually propagated pure lines, differing from one another in one or more allelomorphic pairs, which will serve as the basic material for working out the "chromosome content" in these species. It is only the problem of securing seed from a large number of different localities and of growing and carefully studying a sufficient number of plants that must be solved in order to furnish the pure lines desired. The sooner this can be accomplished the sooner can the extensive analysis of the chromosome content of these species be gotten under way. Finally the critical question as to whether these two species can be hybridized has been answered in the affirmative by the preliminary experiments of the present year.[4]

Sufficient has been said, I trust, to convince the reader that we have in *Crepis* a wealth of material which may fairly be expected to furnish data of the greatest value in testing the generality of the chromosome theory of heredity, and that this group is unique in the promise it holds of carrying out that test in much shorter time than would be required if we should depend only on the data which is slowly accumulating from other plants now under investigation. It should be clearly realized, however, that to accomplish the results aimed at, even with *Crepis*, will require a considerable period of time, the length of the period being largely conditioned by the number of investigators attacking the

[4] Since the above was written difficulty has been encountered in inducing these hybrid seedlings to develop beyond the cotyledon stage. If this difficulty can not be overcome both species will be crossed with still other species having low chromosome numbers.

problem and the facilities at their disposal or, in other words, upon the amount of funds available for this project.

In order to advance the genetic analysis of *Crepis virens* and *C. tectorum* now under way to a stage favorable to carrying out the interspecific hybridization studies properly, calls for greenhouse equipment, technical assistance, supplies and labor which are not at present available. Some provision for the collection of seed in foreign countries should also be made. There is no prospect at this time that these facilities will become available in the near future. It is recognized that the expansion of this project will require a larger proportion of the time of the two investigators now engaged on it and the workers concerned stand ready to meet this requirement.

My purpose in going thus into detail is two-fold. First, so far as I am aware, no other geneticists are working extensively with this genus, and it should be clearly understood that under existing circumstances there is little prospect of rapid progress with my own investigations. Yet the work has gone far enough to accumulate material of very great promise. It is hoped, therefore, that means will be found to support adequately the investigations of *Crepis virens* and *C. tectorum* now under way. Second, it is highly desirable that other geneticists also contribute to the analysis of the two species named above and especially that they proceed with similar investigations, accompanied of course with cytological studies, on other species of *Crepis*.

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## THE INHERITANCE OF CONGENITAL CATARACT IN CATTLE[1]

Cataract in mammals may be due to environmental causes, or it may be hereditary. The mode of inheritance has been debated. In the case of man Bateson (1) and Davenport (2) regarded cataract as a dominant Mendelian character, while Jones and Mason (3) in an analysis of human pedigrees collected by Harmon (4) concluded that cataract is probably a simple recessive. Danforth (5) raised some pertinent objections to this latter hypothesis, and Jones and Mason (6) later admitted the validity of some of these objections. There are a number of elements in this analysis of human pedigrees which are no doubt perplexing, but the preponderance of evidence seems to favor the hypothesis that cataract in man is a Mendelian recessive.[2] Hereditary cat-

[1] Paper No. 10 from the Laboratory of Genetics, Illinois Agricultural Experiment Station.

[2] The data as analyzed by Jones and Mason seem convincing because of the large value of $P$, a measure of goodness of fit of the observed to the calculated series. While there can be little doubt but that the observed results lie within reasonable limits of error when tested by any one of several approved methods, it should, perhaps, be stated that Jones and Mason's use of Pearson's criterion is hardly justified, inasmuch as Pearson's formula applies to a correlated system of variables in which the sum of the observed frequencies = sum of calculated frequencies, and the sum of the errors = 0. Harris (7) pointed out the value of Pearson's formula in relation to Mendelian ratios. Now in any complex Mendelian ratio of the form $(\frac{1}{2}+\frac{1}{2})^n$ or $(\frac{3}{4}+\frac{1}{4})^n$ where $n$ = the number of allelomorphic pairs and where all terms of the ratio are given, the above conditions are always met. However, the observed series which Jones and Mason attempted to fit to a calculated series is neither a complete Mendelian ratio nor does it fulfill the conditions stated. Jones and Mason were not satisfied with the goodness-of-fit value when the heterozygotes were mated *inter se* ($P = 0.418$), but if the deviations were due to random sampling we should expect the values of $P$ to fluctuate around 0.5. Their results would therefore be interpreted as consistent with their theory, if their method were correct. By using the method adopted by Jones and Mason, one might nevertheless obtain a better (?) fit in this case and thus a more satisfactory result if one dealt with the series of normals in this population rather than the series of cataractous. In any monohybrid ratio, the deviation of the dominant class is equal to the deviation of the recessive class. Hence, if we divide the same series of deviations all the way through by a series of larger calculated values for normals, then $X^2$ will be perceptibly smaller and P larger ($P = 0.71$ in this case). This procedure would be somewhat comparable to stating that in a single toss of 8 coins, 5 heads are more likely to appear than 3 tails, or in a single throw of n coins (n–p) heads are more probable than p tails.

aract is known in some mammals other than man, but little is known regarding its transmission. Hurst (8) stated that liability to cataract-blindness in horses is a Mendelian character.

The data forming the basis of this paper arose through the circumstance that a registered Holstein-Friesan bull, E. T. H. (Holstein-Friesan Herdbook No. 62924) transmitted desirable economic dairy characters to a marked degree, and consequently attempts were made to fix his characters by inbreeding. A number of cataractous offspring resulted from these close matings. The simultaneous occurrence of several cataracts in the progeny of a single bull could not be attributed to chance environmental or intrauterine conditions; hence the pedigrees of all animals involved were carefully studied. There was no record of cataract in any of the ascendants. The original bull, E. T. H., had been mated to a large number of unrelated cows and produced 93 normal $F_1$ offspring. Thirty-two of these $F_1$ daughters were mated to an $F_1$ son and produced 63 $F_2$ calves, of which 55 were normal and 8 showed well-defined congenital cataracts of the stellate type.[3] The ophthalmological aspects of these cases have already been described by Small (8).

Cataract is evidently *recessive* in cattle and if it is a simple Mendelian recessive then the original progenitor, E. T. H., was heterozygous, Nn, where N = normal and n = cataractous. Mated to unrelated normal females, NN, we should expect the 93 $F_1$ offspring to be perfectly normal, but of two genetic types in equal numbers, NN + Nn. In selecting any $F_1$ son to breed to the $F_1$ daughters, it was equally probable that he would be either a homozygous normal, NN, or a heterozygous normal, Nn. A single $F_1$ son, also a registered Holstein-Friesan bull, V. H. (Holstein-Friesan Herdbook No. 158293), was chosen and he proved to be heterozygous. Since half of the $F_1$ daughters were homozygous and half were heterozygous, the former would produce gametes, N + N, and the latter would produce gametes, N + n. The total population of $F_1$ daughters would therefore produce three times as many normal as cataractous gametes. In Mendelian notation the $F_1$ matings were as follows:

$$\tfrac{3}{4}N + \tfrac{1}{4}n = \text{gametes from } F_1 \text{ females,}$$

[3] The writers are indebted to Dr. C. P. Small, of Chicago, Illinois, for identifying the type of cataract and for much additional information, and wish to express their appreciation of the deep interest Dr. Small has shown in this case.

$$\tfrac{1}{2}N + \tfrac{1}{2}n = \text{gametes from } F_1 \text{ males,}$$

$$\underbrace{\tfrac{3}{8}NN + \tfrac{4}{8}Nn}_{\text{normal}} + \underbrace{\tfrac{1}{8}nn}_{\text{cataractous}} = F_2 \text{ zygotes.}$$

That is, 7/8 of the $F_2$ calves should be normal and 1/8 should be cataractous. The observed results agree with the calculated rather better than one would usually expect, for the observed = 55 normal + 8 cataractous and the calculated = 55.125 normal + 7.875 cataractous. The 8 $F_2$ cataractous calves were of both sexes (2 heifers and 6 bulls).

All these facts clearly indicate that the original sire, E. T. H., was heterozygous, Nn. If mated to his own daughters he should give results similar to those of his son, V. H. He was thus tested and produced 7 offspring of which 3 (1 bull and 2 heifers) were cataractous. Collecting all matings of the sire, E. T. H., and his son, V. H. (both were Nn) to $F_1$ daughters (NN + Nn) we found:

| | Normal | Cataractous | Total |
|---|---|---|---|
| Observed | 59 | 11 | 70 |
| Calculated | 61.25 | 8.75 | 70 |

We may therefore conclude that congenital cataract in cattle is a simple recessive Mendelian character.

To prevent the reappearance of cataractous individuals in this stock and to reduce the proportion carrying cataract as a recessive is a matter of some economic importance. After all cataractous individuals have been eliminated, there still remain one half of the daughters and four sevenths of the granddaughters of E .T. H. which carry a recessive factor for cataract. We can not distinguish between the NN and Nn individuals, since N is evidently dominant to n. Whatever the proportion of these two types may be to each other, mating the cows to normal unrelated bulls (which are almost unquestionably NN) will eventually reduce the cataractous-bearing individuals to a negligible minimum. If we begin with r + s individuals of the genetic constitutions NN and Nn respectively, and we back cross to normal stock, NN, any number of times, p, then the genetic composition of the last generation produced after p such back crosses will be

$$[2^p r + (2^p - 1)s]NN + sNn.$$

Since the first term becomes much larger than the second as p increases, the number of homozygous normals becomes very great compared with the heterozygous normals.

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## FURTHER OBSERVATIONS ON SEX IN MERCURIALIS ANNUA

In an earlier paper,[1] I briefly mentioned the occurrence of so-called monœcious forms in *Mercurialis annua.* I have since then continued my studies upon such forms and this report deals with the offspring of the so-called monœcious plant, No. 3. It is to be noted that *Mercurialis annua* is described as appearing in

[1] Inheritance of Sex in *Mercurialis annua, American Journal of Botany,* Dec., 1919.

three forms, male, female and monœcious. Several hundred seeds from plant No. 3 were sown in Fargo, North Dakota, in the spring of 1919. Owing to a protracted drought only four plants survived.

In their general habit of growth these plants were like the females of my earlier observations—the flowers were clustered

FIG. 1. Branch *Mercurialis annua*. *a*, male flower bud; *b*, female flower bud; *c*, female flower; *d*, male flower; *e*, hermaphrodite flower.

in the axils of the leaves, either sessile or on more or less elongated peduncles. In another paper (Mss.) I have described in detail the various floral arrangements that appeared on these plants. Female flower buds are conical. The male buds are smaller than the female buds and they are spherical. The hermaphrodite flower buds are like the female buds though sometimes smaller. Just prior to the opening of the hermaphrodite

flower buds, the anthers may be recognized through the sepals. These four plants are not monœcious, since male, hermaphrodite and female flowers appeared simultaneously on the same plant.

Plant No. 3–1 made a vigorous growth from the beginning. Its foliage was dark green. The first flowers were female and these were produced in increasing numbers. No attempt was made to count the female flowers prior to the appearance of male and hermaphrodite flowers. As can be seen from the table the female flowers always outnumbered the male and hermaphrodite flowers. Until September 10 the male flowers were more abundant than the hermaphrodite. On September 12, there was a sudden increase in the number of hermaphrodite flowers. This rather sporadic appearance of flowers other than female flowers shows how impossible it is to determine at one time the sex of the individual. It is apparent that it is essential that such individuals be studied throughout their whole life history. Thus through the first three months of its history this plant was female, after that it was polygamous, monœcious, and gynomonœcious. It was polygamous when beside the female flowers there appeared male and hermaphrodite flowers, monœcious when only male flowers appeared in association with the female flowers, and gynomonœcious when hermaphrodite flowers appeared together with female flowers. The total number of male and hermaphrodite flowers was about equal (table). This plant may be characterized as a polygamous one.

While there were no definite points at which male or hermaphrodite flowers appeared, there were branches that continued to produce only female flowers throughout the life of the plant. Thousands of seeds were collected from the plant.

Plant No. 3–2 like plant No. 1 made a vigorous growth. Its foliage was much lighter than that of plant No. 1 but the plant was healthy. As can be seen from the table the number of male and hermaphrodite flowers that appeared at one time was relatively larger than in any of the other plants. This condition was maintained throughout the life of the plant. This plant from the time of the appearance of male and hermaphrodite flowers was decidedly polygamous, prior to that it produced female flowers like plant No. 1. During the period in which the three kinds of flowers were counted, female, male, and hermaphrodite, the male flowers were in excess. It may be conceived

then that during a part of its life history the male elements predominated. This plant was a very prolific seed producer.

Plant No. 3–3 was a very vigorous grower and it behaved like plants Nos. 1 and 2 until the time of the appearance of male and hermaphrodite flowers. The total number of male flowers when compared with the total number of hermaphrodite flowers showed that the tendency of the plant was towards monœcism. While during most of its later history male and hermaphrodite flowers appeared together, towards the end of the growing season (October 3–12) no hermaphrodite flowers were found and the plant was decidedly monœcious. This plant started out as a female, became polygamous and towards the end became monœcious. Many seed were set.

Plant No. 3–4 started out as a vigorous plant producing in the beginning female flowers in abundance. About the same time that the other plants were producing increasingly large numbers of male and hermaphrodite flowers this plant produced very few, 10 males and 4 hermaphrodites. After that the plant began noticeably to lose in vigor, the leaves began to curl up. The plant after that produced female flowers in abundance. These however dried up very quickly and dropped off. The plant continued its sickly growth until it was killed by frost.

Pistillody and staminody occurred very abundantly in the flowers of the first three plants. This condition I have described in detail in another paper (Mss.). Many of the hermaphrodite flowers had only a single stamen. The plants also produced a large number of three-carpelled female and hermaphrodite flowers whereas a two-carpelled flower is the rule.

While the number of plants is too small to warrant the drawing of any definite conclusions the following suggestive facts are brought out.

1. Sex is not a fixed condition in these forms of *Mercurialis annua*.

2. A plant may change its sex during the progress of its life cycle.

3. Continued study with larger numbers of such plants will very likely show marked variations and sex intergradations and that a strict category of sex for these forms is untenable, so that the terms monœcious, gynomonœcious, gynodiœcious, etc., can be only arbitrarily employed.

| Plant | May 25 to Aug. 25 | Aug. 27 | | | Aug. 30 | | | Sept. 1 | | | Sept. 4 | | | Sept. 8 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Only ♀ Flowers Produced | ♀ Fls. | ♂ Fls. | ☿ Fls. | ♀ Fls. | ♂ Fls. | ☿ Fls. | ♀ Fls. | ♂ Fls. | ☿ Fls. | ♀ Fls. | ♂ Fls. | ☿ Fls. | ♀ Fls. | ♂ Fls. | ☿ Fls. |
| 3–1 | ∞ | ∞ | 11 | 2 | ∞ | 4 | — | ∞ | 5 | 1 | ∞ | 4 | 6 | ∞ | 40 | 10 |
| 3–2 | ∞ | ∞ | 25 | 6 | ∞ | 70 | 36 | ∞ | 294 | 298 | ∞ | 158 | 163 | ∞ | 282 | 126 |
| 3–3 | ∞ | ∞ | 5 | 1 | ∞ | 3 | 4 | ∞ | 2 | — | ∞ | 9 | 4 | ∞ | 15 | 9 |
| 3–4 | ∞ | ∞ | — | — | ∞ | 1 | — | ∞ | 6 | 4 | ∞ | — | — | ∞ | 3 | — |

| Plant | Sept. 10 | | | Sept. 12 | | | Sept. 16 | | | Sept. 18 | | | Sept. 22 | | | Sept. 25 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ♀ Fls. | ♂ Fls. | ☿ Fls. | ♀ Fls. | ♂ Fls. | ☿ Fls. | ♀ Fls. | ♂ Fls. | ☿ Fls. | ♀ Fls. | ♂ Fls. | ☿ Fls. | ♀ Fls. | ♂ Fls. | ☿ Fls. | ♀ Fls. | ♂ Fls. | ☿ Fls. |
| 3–1 | ∞ | 18 | 8 | ∞ | — | 150 | 580 | 50 | 40 | | | | 120 | 40 | — | 200 | 20 | 100 |
| 3–2 | 750 | 700 | 450 | 750 | 850 | 550 | 590 | 650 | 350 | 301 | 550 | 512 | | | | 165 | 225 | 60 |
| 3–3 | ∞ | 22 | 4 | 220 | 220 | 60 | ? | 20 | ? | 60 | 200 | 50 | 120 | 340 | — | 180 | 110 | 30 |
| 3–4 | | | | | | | | | | | | | | | | | | |

| Plant | Sept. 27 | | | Oct. 3 | | | Oct. 12 | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ♀ Fls. | ♂ Fls. | ☿ Fls. | ♀ Fls. | ♂ Fls. | ☿ Fls. | ♀ Fls. | ♂ Fls. | ☿ Fls. | ♀ Fls. | ♂ Fls. | ☿ Fls. |
| 3–1 | 102 | 91 | 24 | 500 | 100 | — | 1000 | 200 | 200 | ∞+2502 | 583 | 541 |
| 3–2 | 130 | 130 | 80 | 400 | 480 | 500 | 360 | 480 | 120 | ∞+3446 | 4892 | 3251 |
| 3–3 | 340 | 360 | 20 | 200 | 50 | — | 800 | 400 | — | ∞+1920 | 1906 | 182 |
| 3–4 | | | | | | | | | | ∞ | 10 | 4 |

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## COMMENTS ON A RECENT CHECK-LIST

RESEARCH stations established in the past by scientific institutions, especially those in or near the tropics have generally been devoted particularly to study of aquatic organisms. It was, therefore, with great pleasure and with high hopes for its future that naturalists all the world over have watched with keenest interest the establishment and gradual development of the Tropical Research Station of the New York Zoological Society.

Mr. Beebe has shown great acumen in selecting his locality. His facile pen has drawn the wonders of his station's environment in a way so splendidly vivid that I, for one, envy very frankly his skill and his good fortune. These comments then are offered here, on one of his recent papers, with a cordial appreciation of the debt which all naturalists owe to him for what should in the future become the most useful workshop of its kind: indeed to be thought of always in future as bearing a relation to the tropic rain-forest in the same way that one subconciously recalls the Naples Station when thinking of or discussing the fauna of the Mediterranean Sea.

Beebe has charmed many readers with essays which show that he is gifted with a delightful diction and a romantic style most convincing and hence to be most carefully used. To criticize these essays unkindly is far too much like picking apart an orchid. Nevertheless they sometimes have the defect of capitalizing supposed "new discoveries" at a rather high advertising value when the history of our earlier knowledge has not been determined from the literature.

Thomas Penard, in an article of marked gentleness and courtesy,[1] has reviewed Beebe's "Tropical Wild Life," in such a way that further elaboration is happily unnecessary. Now, however, articles have appeared in *Zoologica*,[2] which require more careful examination. They purport to be usable lists, admitted to be necessary, for any study of the higher vertebrates of British Guiana with special reference to the fauna of the Bartica district—the species which Beebe has actually found there being starred with an asterisk.

Beebe introduces them as follows:

> Finding no résumé available of the Amphibia, Reptilia and Mammalia of this colony, I have gone through the literature at hand and made my own lists. These I offer as a preliminary enumeration of the species thus far recorded in literature, or in my own collections, from this British Colony. They form a tangible basis for future increments—the many new species and the radical extension of present known distributions which intensive study of these phyla in British Guiana is certain to achieve. *Check-lists of mere names such as these are wholly foreign to the future zoological work of the Tropical Station* (italics mine), but they are *absolutely necessary* as a basis for identification and investigation, and it is in this spirit that this preliminary work has been undertaken.
>
> I have made no attempt at a thorough search of literature for priority or for confirmation of names or other similar phases of taxonomy, deeming this the special province of the literary systematist. I have merely sought to utilize the most recently accepted names of herpetologists and mammalogists.

Now, generally speaking, the "literary systematist" does not confine himself to this somewhat dry but entirely necessary vocation, wholly from choice and he is saddened when his more footfree colleagues cast supercilious glances his way. It therefore

[1] *Auk*, 36, 1919, pp. 217–225.

[2] Vol. 2, Nos. 7 and 8, 1919.

behooves the now deservedly but still very highly vaunted "FIELD NATURALIST" to write with care when he deliberately invades the province of his more lowly associate. Systematists know that the preparation of a good, useful check-list of a region like British Guiana is no task to be entered upon lightly nor unadvisedly and because the work is not spectacular, arouses little popular acclaim and is slow and tiresome, we sometimes wonder whether these facts have not a high value in explaining the rather supercilious and scornful appraisal given to a mere check-list by the modern field naturalist. Admittedly, however, this work if worth doing at all, is worth doing well but this list of Beebe's is so phenomenally bad that we are loath to believe that Mr. Beebe has tried to make it even moderately good. For example, in so far as reptiles and amphibians—or mammals—are concerned there is no evidence that specimens of many of the obscure species discussed have ever been preserved for examination by a herpetologist.

We read of *Bufo molitor*. This name was given by Tschudi[3] to a toad from high Peru. Naturalists in recent years have so far as I know felt reasonably sure that this was a synonym of *Bufo marinus* pure and simple. Stejneger has said recently:

> Whatever may be the status of Tschudi's *Bufo molitor* the half grown toad [taken by the Yale Peruvian Exp.] collected at Santa Ana . . . unquestionably belongs to *Bufo marinus*.

So also all the Peruvian examples collected by Mr. G. K. Noble and now in the M. C. Z., Cambridge. Here then this name appears resurrected in literature and recorded from Bartica, of all places, and no proof whatsoever offered to support so wholly improbable a statement.

*Bufo sternosignatus* Keferst. The types came from western Venezuela and Colombia. Günther's figure of this species shows how easily it also might be taken for the young *Bufo marinus*. Since apparently the species is not known from eastern South America can this be called a valid record until Beebe's specimen, if it was preserved, falls into a herpetologist's hands?

*Hyla indris* Cope. Another species, known apparently only from Cope's original description which strongly suggests that it was probably nothing but an individual variant of *Hyla crepitans*.

[3] Fauna Peru, Herp., 1845, p. 73, Pl. 12.

*Hyla punctata* of Beebe's list is probably *Hyla helenæ* Ruthven, described *from British Guiana* and evidently *entirely unknown* to Beebe.

*Hyla fasciata* here definitely recorded from British Guiana although not captured (no asterisk), hence the record is probably copied from Boulenger's Catalogue, where there is a large question mark which is here omitted.

*Hyla lineomaculata* Werner is yet to be proved distinct from *Hyla rubra.*

The *Ceratophrys cornuta* starred as having actually been taken would indeed have been a prize had it fallen into appreciative hands. For the finding of this species so far from home would be worth most painstaking verification. Has Mr. Beebe saved the specimen? It is not in the American Museum in New York, whose reptile series suffers sadly through Beebe's scorn of the collector.

Specimens must be seen before the records of *Leptodactylus longirostris* Boulenger (type locality Santarem), *Leptodactylus ocellatus* (Linné), widespread in the southern South American grasslands and *Leptodactylus gaudichaudii* can be considered.

Suddenly using capitals for specific names, perhaps in a burst of enthusiasm at the shock which he knows the "closet naturalist" will suffer, we read that he has found Otophryne "Robusta" at Bartica, so also Atelopus "Proboscideus," Atelopus varius and Atelopus pulcher. In the same category of most highly improbable records, among others, we find *Anolis ortonii* of the Peruvian montaña and *Anolis sagrei* a native of Cuba and the Bahamas. *Ameiva surinamensis* is referred to in an adjoining paper correctly as *Ameiva ameiva,* we wonder if they are considered the same species. *Prionodactylus* we had always supposed to be a characteristically Andean genus yet here the Equadorian oshaughnessyi appears as actually occurring at Bartica! *Cophias* should appear as *Bachia* but Beebe would probably consider this as "in the special province of the literary systematist" or is this simply a case of where Mr. Tee-Van, in his "untiring search" of the literature, got too tired before the pertinent reference was found? The boas' names are rather confused as we use them now—another purely literary matter, however. The nomenclature of the snakes in general is a mixture of earlier usage with the acceptance of such a radical concession to necessity as the use of *Micrurus* for *Elaps,* while in many other cases

no attempt has been made to follow the now generally accepted canons of the International Code of Zoological Nomenclature.

It is hard to review a paper of this sort without being personal, for personality becomes most inexplicably pushed into what at first sight is pure dry-as-dust. It is only fair to offer constructive criticism also in such a case as this. Beebe should first learn the value and importance—and the use—of an adequate library. He should have attached to his staff trained taxonomists who are also skillful collectors. These men should, taking the fauna group by group, make careful determinations so that the observers at the station may know what they are working with. Any reliance in the future on such a list as the one published—admitted to be necessary—yet "wholly foreign" to the station's aim—will be regarded by sincere naturalists with pity at the great opportunity lost and sorrow at the misuse of resources and energy.

We may point our moral and adorn our tale with the wish that: when that "little Danish flapper" in St. Thomas taught Beebe that lizards may be noosed as he tells us, "Thus after years of effort" we wish that instead of only showing him what every reptile collector learns from the first urchin he meets, if he has not already devised the scheme by instinct, be the urchin yellow, red, white or black, that she had said "Oh, kind Sir! Do *keep the lizard* for your less happy colleagues at home will still have much to learn from that poor despised little pickled carcase."

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# INHERITANCE OF CALLOSITIES IN THE OSTRICH

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"*The problem of the method of evolution is one which the biologist finds it impossible to leave alone, although the longer he works at it, the farther its solution fades into the distance. The central point in the problem is the appearance, nature, and origin of the heritable varieties that arise in organisms.*"—H. S. JENNINGS.[2]

THE ostrich has a shield-like sternum devoid of a keel, a character it shares with the rest of the Ratitæ. The middle forms a broad, rounded projection, while the covering skin is greatly thickened, devoid of feathers, and constitutes a large, dense callosity on which the bird rests when crouching. Moreover, the ostrich is unique among birds in having a symphysis pubis, which forms a ventral projection behind corresponding with the one in front, only smaller, the skin over it likewise showing a strong callosity (Fig. 1). The result is that when the bird crouches the two median projections come into direct contact with the ground and the thickened pads support the greater part of the weight of the body, about 250 lbs., in front and behind, while it is steadied laterally by resting upon the upper surface of the nearly horizontal meta-

[1] The author is indebted to Dr. Raymond Pearl for seeing the paper through the press.

[2] *Journ. Washington Academy of Sciences*, Vol. VII, No. 10, May 19, 1917, p. 281.

tarsals and feet (Fig. 2). The sternal and pubic callosities may therefore be looked upon as a direct response of the skin to the pressure and friction of the body against the hard ground. Also in its frequent habit of

FIG. 1. Under surface of ostrich showing the large sternal callosity in front and the small pubic callosity behind. The darkened surface of both is due to the adherence of dirt. The bird is a young cock about eighteen months old in which the white ventral feathers are not yet completely replaced by black.

taking a "dust-bath" the ostrich rolls from side to side, the two projections being in the axis of motion, and this serves further to extend the area subject to pressure and friction.

In man and mammals generally a callosity usually con-

sists of a single, smooth or papillose thickened area of the skin, resting upon a bony support; but in the ostrich, as in other birds and in reptiles, it is constituted of a number of separate and distinct thickenings, somewhat regular in their arrangement, which give the appearance of a rounded or angular mosaic or tessellation (Figs. 5 and 6). This is typically shown on the under surface of the toes of birds and lizards, where the elements tend to

FIG. 2. Group of young ostriches, about six months old, the one in the foreground seen in a half-crouching attitude. The weight of the body is supported upon the inside of the ankle and the partly upturned two toes. When fully crouching the bird lurches forwards and comes to rest upon the sternal and pubic callosities, the tarsus and toes remaining in the same position.

be elongated and present a coarsely villous effect. Where the skin is scaly each callous constituent corresponds with an individual scale, but the latter has evidently no determining influence upon the form assumed, for the same tessellated arrangement is found over the sternal and pubic thickenings, though no scales are present. It is probable that the typical form of the reptilian callosity was first determined by the presence of the epidermal scales of the skin, and the latter still responds in the same manner in birds, not only on the legs and toes where scales occur, but over other parts of the body from which they are absent. The present interest lies in the fact that the characteristic form assumed by a callous area in the ostrich enables it to be sharply distinguished from the

surrounding parts of the skin which remain smooth. The tessellation, along with the thickening below, gives it a distinctive character as compared with the pads in mammals, which are mere thickening of the skin, and whose claim to be regarded as a "character" might at times be disputed. Where a callosity assumes any considerable thickness the underlying bone exhibits a correlated response by likewise becoming thickened, as is well shown on both the sternum and pubis of the ostrich.

The skin of all vertebrates appears to have the inherent power of responding to frequently repeated pressure and friction by the formation of thickenings over the bony projections upon which it rests. The pads are special protective adaptations to meet intermittent pressure and friction, upon what would otherwise be soft vulnerable parts of the body. They can arise at any part of the surface of the skin and may slowly disappear when the causal stimuli are no longer operative. Many of them are temporary responses, acquired during a part of the life-time of the individual, and come under the group of adaptive somatic modifications which are non-transmissible, though others, especially those on the under-surface of the feet, are transmissible and may therefore be regarded as germinal in their origin. *Thus similar characters, alike in structure and function, may be either individually acquired and non-transmissible or germinal and heritable.*

The ostrich resembles man and other animals in having the inherent power to produce special callosities over parts of the skin not usually subjected to pressure and friction, as the following observation proves. A chick was hatched in the incubator with its legs widely apart, in such a manner as to be incapable of supporting itself upright in the normal fashion. A deformity of this nature is not unusual among both ostrich and poultry chicks as a result of imperfect incubation, but can generally be rectified by bandaging the legs and drawing them nearer together for a day or two. In this instance however advantage was taken of the deformity to deter-

mine how far the skin would respond to unusual friction and pressure. With its legs widely apart, the chick naturally lay almost prone upon the ground, the inner side of the ankle constituting a feeble support, the tarso-metatarsus having here a projecting knob. The chick was able to raise itself slightly upon the latter and also to drag itself along the ground. It was kept alive for about ten days, and in that time developed a very conspicuous callous thickening over the inside of the metatarsal knob just below the ankle, the normal hereditary callosity along the back of the ankle being unused. The thickening was covered with the minute scales present over the leg generally, but the degree of friction was too intense and continuous for the skin wholly to adapt itself, and a slight abrasion occurred at the apex of the thickening, as in the human hand where pressure and friction are applied too continuously for the callous formation to keep pace with them.

The sternal and pubic callosities are not the only ones in the ostrich which appear to represent adaptive responses to the special habits of the bird. When taking its frequent sand-baths, it rolls about in the dry sand or dust, from side to side, and at the same time uses its wings in an oar-like manner. During the process the under surface of the latter is dragged over the ground and then turned upwards, inwards and backwards, scattering the sand or dust over the body generally, first from one wing and then from the other. The front or pre-axial border of the wing is necessarily subjected to much friction, and develops slight callous areas wherever the internal bones project. Further, the third digit of the wing, which is usually buried in the flesh, is occasionally found projecting freely from the under surface, and its tip naturally comes in for a good deal of rough wear as the latter is dragged along the ground. In response, it becomes knob-like and thickened, the surface showing the characteristic callous markings (Fig. 3). The free tip of the supporting phalanx is also knobbed.

Taking into account the responsive nature of the skin

along with the activities of the ostrich there appears no reason why the sternal, pubic and alar callosities should not be regarded as direct, structural responses to the pressure and friction to which these parts of the body are subject in the every-day activities of the bird. They could be understood as acquired, adaptive characters. The experiment given has served to prove, what would naturally be expected from experience with other animals, that the skin generally is endowed with the power

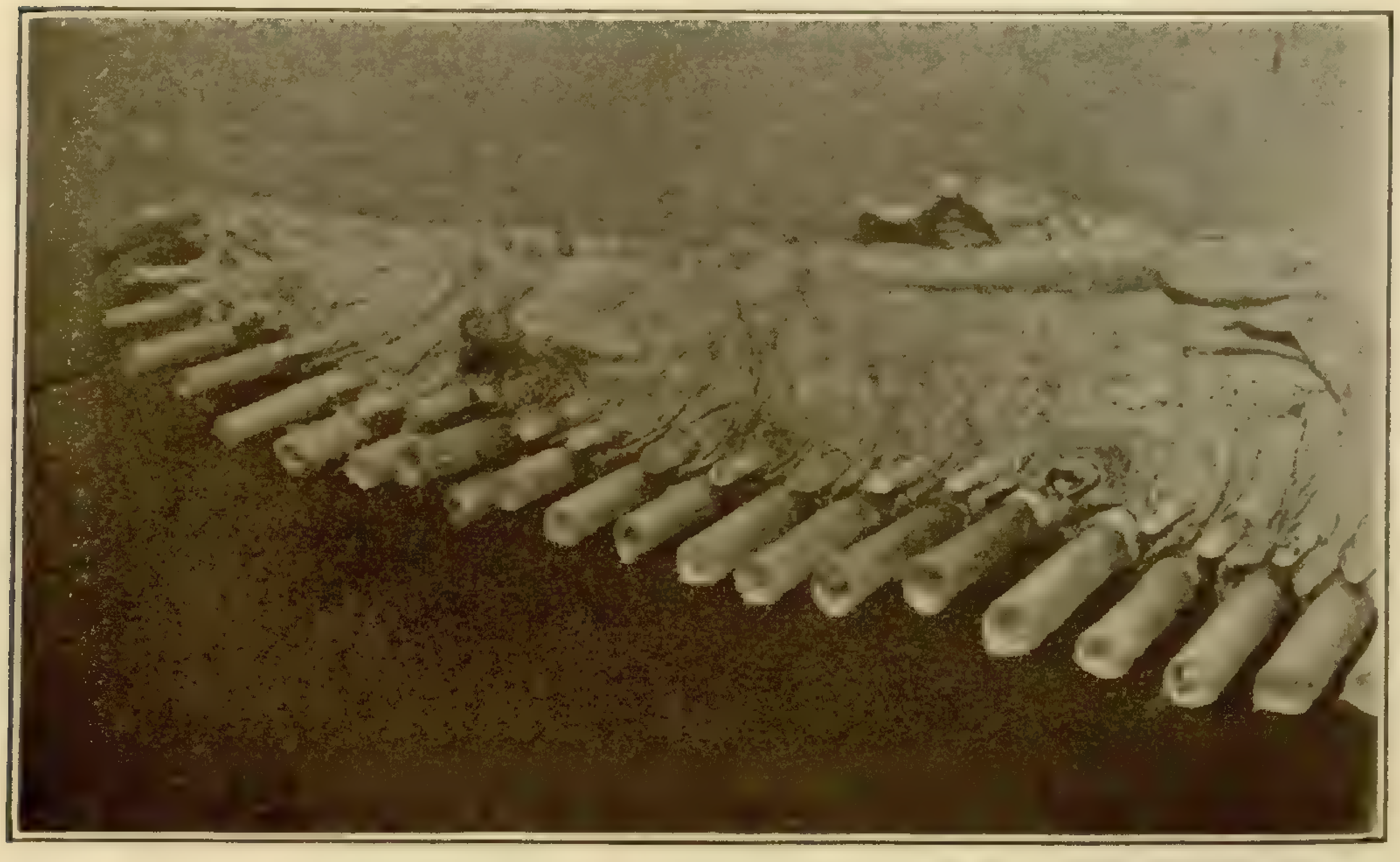

FIG. 3. Under surface of wing showing projecting third digit. The clawed ala spuria is seen above, the second finger is axial and also clawed, while the third projects freely from the under surface and is callous and knobbed.

to make callous responses when subjected to the necessary stimuli.

It was with some surprise therefore that in a series of embryos, representing all the stages passed through during the 42 days of incubation of the ostrich, the later ones were found to possess a perfectly developed callosity over both the sternum and the pubis, of exactly the same form and nature as in the young chick and adult (Fig. 4). The papillary outlines shown to be such a characteristic feature of sauropsidan callosities have the same variations in size and distribution as in the adult, and serve clearly to delimit the callous area from the remaining smooth

surface of the body. Examination of chicks from the time of hatching onwards leaves no doubt that the prenatal callosities become those of the adult, the elevations becoming larger and coarser with use.

The rather insignificant callosities on the wing also show themselves on unhatched and newly hatched chicks.

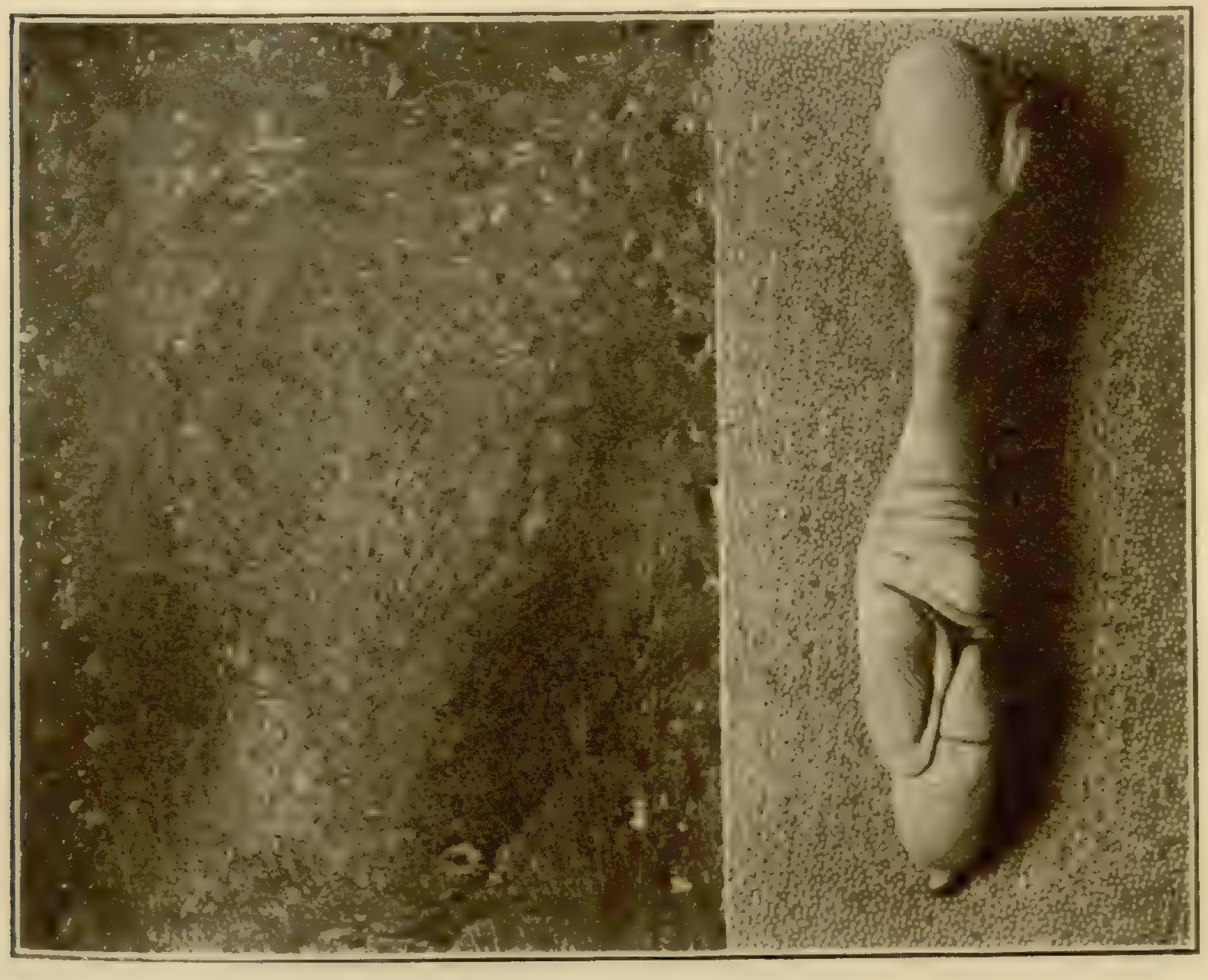

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FIG. 4. Sternal covering of ostrich chick two or three weeks after hatching, showing the hereditary callosity fully established and functional. The cut ends of the feathers are seen surrounding the naked area.

FIG. 5. Callosities on foot and ankle of ostrich chick a few days before hatching. The thickenings are already well developed, the separate elevations on the toes being much narrower and closer than those on the ankle.

They are hardly distinguishable by any special thickening of the skin, but by the appearance of a faint reticulation in places corresponding with those in which they are found in the adults, and which serves clearly to separate them from the surrounding smooth surface. Even the tip of the third digit where sufficiently projecting shows a few markings, leaving no doubt they would later become the functional callosity.

We have therefore in the ostrich certain hereditary structural characters whose independent formation could in every respect be accounted for during the life-time of the bird from the known responsive nature of the skin and the habits of the creature. Examination of the adult alone and a knowledge of its activities would have justified us in regarding them as acquired adaptive characters, had not observation proved that they appear on the chick prior to hatching, and before the parts could have been subjected to the usual stimuli. *The ostrich has hereditary characters which could also be produced as adaptive responses to the habits of the bird.*

The old contentious question therefore arises as to whether the character first appeared as a response of the skin to the habits of the ostrich and has now become hereditary, or whether, having arisen fortuitously in the germ plasm, wholly apart from any adaptive need of the bird, it is now utilized by it. Has the habit developed the character until it has become transmissible or, the character being given, has it permitted of the adoption of the habit? The reply is simple and free from doubt: the callosity under any circumstances would develop *pari passu* with the habit and need of the bird, and neither the callosity nor the habit is dependent upon any antecedent formation. If the character did not arise in the first instance from the activities of the bird, subsequently becoming transmissible, it is manifest that it could originate by two distinct and independent methods, namely, from the germ-plasm and from post-natal stimuli.

It is not the first time that the presence of callosities in the embryos of animals provided with them in the adult has been adduced as evidence that characters originating during the life-time may be transmitted to the offspring. The best known case is that of the wart-hog, another African type—*Ex Africa semper aliquid novi.* With reference to this Professor J. Arthur Thomson[3] remarks:

[3] "Heredity," London, 1912, p. 180.

The African wart-hog (*Phacochœrus*) has the peculiar habit of kneeling down on its fore-limbs as it routs with its huge tusks in the ground and pushes itself forward with its hind-limbs. It has strong horny callosities protecting the surfaces on which it kneels, and these are seen even in the embryos. This seems to some naturalists to be a satisfactory proof of the inheritance of an acquired character. It is to others simply an instance of an adaptive peculiarity of germinal origin wrought out by natural selection.

In the latter part of the above quotation Thomson merely presents the two opposing views without affording us the advantage of his own. The last sentence is a succinct expression of present-day orthodoxy, and we may well consider how far it is justifiable in the case of the ostrich. It is manifest that from their very nature the callosities are outside the realm of competitive strife, and therefore could not have been "wrought out by natural selection." If a character is such that it must perforce be produced as a result of the every-day activities of an animal it is as wholly gratuitous to invoke natural selection as it would be to seek an independent germinal origin. As already shown, the skin of the ostrich is of such a nature that it will form callosities wherever friction and pressure are intermittently applied, just as surely as they will be produced on the human hand as a result of manual labor, on the finger tips of the harpist, violinist or rosary devotee, or on toes encased in ill-fitting boots, with all of which natural selection has no concern. Originally natural selection may have been operative in the survival of animals having the inherent power to form the thickenings, but we have abundant evidence that all the higher forms now possess it.

When the ancestral ostrich first took to resting on its sternum and pubis and rocking from side to side, the callous thickenings would arise quite apart from any antecedent formation and whether or not the germ-plasm had anticipated the need. An inherent power is transmitted, and nothing is gained by transmitting the callosities themselves, since they are adaptations which could arise in the natural course as needed. No selection

is involved in producing the "horny hand of toil"; it forms in the individual in proportion to the need for it. If fore-doomed to hard manual labor some advantage may possibly be conceived in having the callosities in advance, but would be insufficient to be of any selection value.

The position resolves itself as follows: From the known responsiveness of the skin of the ostrich to intermittent pressure and friction and the established activities of the bird it is just as certain that the sternal, pubic and alar callosities could be acquired in each generation independently as that similar thickenings could develop on the palm of the human hand engaged in labor. If we are not prepared to admit that the callosities first arose as somatic adaptations and then became hereditary, we have to face the alternative that at some time in the history of the ostrich a change took place in its germ plasm of such a nature as to give rise to a directly adaptive character, altogether similar to what could be somatically acquired; we have to admit that an exactly similar character could be produced in two wholly different ways: (*a*) directly as a response to the activities of the bird; (*b*) as a result of germinal changes. The same character could be somatically acquired and could arise germinally.

Of course the same argument could be applied to the strongly marked callosities on the toes and ankle of the ostrich which are also hereditary (Fig. 5). But these are not so peculiarly specific for the present purpose. Hereditary pedal thickenings occur in most animals, and even Darwin[4] regarded the thickened sole of unborn infants as "the inherited effects of pressure during a long series of generations." The thickenings on the sternum, pubis and wings are confined to the ostrich, and therefore afford a more circumscribed case for discussion, hereditary transmission from any other type being placed out of consideration, though it is not unlikely that some of the other Ratites may have corresponding structures.

An acquired, non-transmissible, callous pad, presumably due to a change in the crouching habit of the ostrich

[4] "Descent of Man," p. 18.

in the course of its phylogeny, remains to be noticed, as a further instance of the responsive power of the skin. Near the mesotarsal ankle-joint occurs a strong, elongated, hereditary callosity covering the median part of the broad, proximal end of the tarso-metatarsus (Fig. 5). This pad would naturally be used if the bird rested

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FIG. 6. Ankle region of young ostrich showing the symmetrical hereditary callosity above and the accessory one forming below on the inside (to the left).

FIG. 7. Ankle region of old ostrich in which the accessory ankle callosity (on the right) has become coarse and broken up.

squarely upon its tarsus and foot when crouching, the weight being mainly on the ankle. The ostrich however makes little or no use of it, for even in young chicks scarcely any evidence of contact with the ground can be observed. Somewhat to the inside there appears a new callous thickening, which begins to form by the time the chicks are a month or two old, and remains as the func-

tional pad throughout life, taking the place of the hereditary one which, though hardly used, persists structurally (Figs. 6 and 7). The new callosity is continuous laterally with the old median one, and altogether resembles it in character. No trace of it however appears in the chick prior to hatching (Fig. 5), hence it represents an individually acquired, adaptive character in the truest sense. We have therefore an original part of the ankle callosity which is hereditary, though now non-functional, and an acquired part which is functional and non-transmissible.[5]

The main facts presented seem capable of interpretation only in one of two ways: (*a*) *An acquired character which represents a structural response to stimuli resulting from the activities of the organism may become transmissible.* (*b*) *A character may arise germinally of a form and nature exactly similar to one which would otherwise be acquired independently from the known activities of the organism and the established responsive nature of its structural parts.*

[5] Manifestly in the course of its phylogeny some change has taken place in the manner of crouching of the ostrich, for instead of resting squarely upon the median part of the ankle it has come to support itself mainly upon the inside. One ventures the suggestion that the change is to be associated with the loss of the second toe in the course of the retrogressive evolution of the foot. During a part of its phylogenetic history the ancestral African ostrich had unquestionably three toes like the living *Rhea,* the American three-toed ostrich, representing the second, third and fourth of the pentadactyle series. The second has disappeared in the two-toed ostrich *Struthio,* though considerable traces exist in the embryo.

In its three-toed stage the ostrich would rest squarely upon its ankle, the other extremity of the limb being steadied by the upturned three toes, a smaller one on each side of the large middle third. A symmetrical median callosity would naturally form at the ankle-joint and, according to the view here maintained, would become transmissible. With the loss of the inner or second toe through degeneration the inside distal support for the tarsus would disappear, and the latter would tend to tilt inwardly along its whole length, in such a manner that the median part of the ankle would no longer support the bird. The callosity over it would be unnecessary, but a new one would form over the new area of support. In the ostrich of to-day the ancestral, hereditary ankle callosity, reminiscent of the three-toed stage, still appears, though functionless; a new non-hereditary one is acquired afresh with each generation and assumes the function of the old, becoming the ankle support for the crouching two-toed bird (Fig. 7). The whole forms a remarkable illustration of correlation between a phylogenetic change and an adaptive ontogenetic modification.

In adopting the first interpretation we depart from the generally accepted opinion of biologists of the present day and admit that an acquired character may become transmissible; in maintaining the second we are exercising a credulity unjustified by biological experience.

In the voluminous literature of evolution and heredity, case after case has been brought forward by advocates such as Lamarck and Herbert Spencer, claiming to be illustrations of the inheritance of acquired characters, and just as surely has it seemed possible to interpret them in some other fashion, as Weismann and others have insistently done. The fate which has befallen these should suffice to make the boldest hesitate in adducing yet another. It is the apparently unassailable character of the two opposing statements above which emboldens one in all diffidence to re-open "the interminable question" of the late Professor W. K. Brooks, that leader and inspirer of so much American philosophical biology. The peculiar justification for the present claim seems to be that, were the callosities of the ostrich not transmissible, they could be acquired just as effectively from the responsive nature of the skin of the bird; also that natural selection has no bearing on the question, for they are adaptive structures which the organism has the inherent power to produce as required.

According to Weismann (quoted from Walter[6]) three things are necessary to prove the inheritance of acquired characters: "*first,* a particular somatic character must be called forth by a known external cause; *second,* it must be something new or different from what was already exhibited before, and not be simply the reawakening of a latent germinal character; and *third,* the same particular character must reappear in succeeding generations in the absence of the original external cause which brought the character in question forth." It is contended that all the circumstances surrounding the sternal and pubic callosities of the ostrich are in full accord with these three requirements.

When assuming that an acquired character has become

[6] H. E. Walter, "Genetics," Macmillan & Co., 1913, p. 94.

transmissible it is usually held that in some mysterious fashion it has so impressed itself upon the soma that it becomes represented in the germ plasm by one or more factors, determinants or genes which are able to reproduce the same character in the next generation. The difficulties of conceiving this are so great as to convince most students of its impossibility. On the other hand we have to admit that we know little as to the means by which a germinal factor arises and gains its expression as a somatic character. Apart from the accessory chromosome in sex cells and the highly suggestive work of Professor T. H. Morgan and his associates on germinal *loci* in *Drosophila,* we only know of factorial representation by somatic expression. We are ignorant of the relationships between the two, and of the measures by which one gives rise to the other. Were it not that Thomson has shown the contrast to be hardly justifiable, one would be inclined to ask: Is it not as difficult to understand how a genetic factor arises and comes to have somatic expression as it is to conceive how germinal representation may be gained by an acquired somatic character? We accept the one without demur, but are prone to deny the other as impossible. We must not forget the warning of Professor Lloyd Morgan that because the phenomenon of acquired transmissibility can not be understood it is not necessarily rendered impossible.

In considering the difficulty in the way of an acquired character gaining factorial representation in the germ plasm it is legitimate to enquire whether a transmissible character is necessarily germinal as present-day teaching so consistently affirms, that is, whether it is necessarily represented in the germ plasm by definite genetic factors.[7] We have admitted that we know little or noth-

[7] In a sense everything appearing in the soma may be regarded as derived from the germ, but the factorial hypothesis has given us a clear understanding as to what is meant when we say that a character is germinal. With the question of acquired characters before us there need be no confusion as regards a germinal and a non-germinal character, and whether the latter appears pre-natally or post-natally. On the considerations here set forth a transmissible character is not necessarily represented directly by germinal genetic factors.

ing of the manner in which factorial representation in the germ plasm gains expression in the soma; on the other hand we have some experience, from observation and experiment, of the production of somatic changes in the life-time of the organism, as a result of environmental influences and of stimuli due to the use and disuse of parts. The production of callosities, the variation of muscles and the skeletal changes in correlation therewith, the direct modification of bones, ligaments and mesenteries, are all adaptive changes which may result as responses to the external and internal stimuli to which the organism is subject during its life-time.[8] They reveal the inherent powers of responsive adaptability present in the tissues and organs of the body. They are in truth characters which arise independently of direct representation in the germ plasm, and indicate that the latter is not the *fons et origo* of all structural changes. The power of the tissues to respond to stimuli is transmissible; irritability, the power of responding to stimuli, is one of

[8] In a series of papers appearing in the *Journal of Anatomy and Physiology* from 1886 to 1888, Sir W. Arbuthnot Lane presents a remarkable series of adaptive changes which take place in the human body as a result of continued occupational activities. They are probably the fullest and most complete studies of this nature which we possess. One contribution, "A remarkable Example of the manner in which Pressure-Changes in the Skeleton may Reveal the Labour-History of the Individual," is a full account of the changes which appear in the skeleton of the coal-trimmer. The most notable feature is the formation of an arthrodial joint in the fibro-cartilage between the fourth and fifth lumbar vertebræ and the division of the neural arch of the fourth at two points, a result of the forcible rotation of the spine on a vertical axis which takes place when coal is thrown with great force to a considerable distance, as when the coal-trimmer is engaged at his work on board ship.

A second paper, "The Anatomy and Physiology of the Shoemaker," describes the anatomical and osteological changes which had resulted from the habitual performance of a definite series of movements entailing the expenditure of a considerable amount of muscular energy, during the greater part of a long life-time of seventy-three years. The most striking change is the formation of a buttress of bone, which extends upwards from the lateral mass of the atlas on the one side, and articulates by means of an arthrodial joint with the jugular process of the occipital bone.

Along with other papers dealing with more or less cognate subjects the two are included in a single volume under the non-suggestive title, "The Operative Treatment of Chronic Intestinal Stasis," J. Nesbet & Co., London, 3d ed., 1915.

the fundamental attributes of protoplasm. The manner of the response is adaptive, it is an individual effort, and is usually non-transmissible. Whether the responses ever become transmissible, in that they appear without the original stimulus, is the crucial point of the problem of the transmission of acquired characters. That the organism has the inherent power of forming new non-germinal characters is however not questioned, and it is well that the hard fact should be kept in mind. What we desire is some evidence that stimuli are transmissible or, if this be not forthcoming, some proof that the responses may appear without the original stimuli. At first this may be deemed to be looking for an effect without a cause, a response without a stimulus.

The callosities in the ostrich and adaptive responses generally lead one to submit that a character may become transmissible without necessarily being germinal, in the sense of having factorial representation in the germ plasm. Acquired characters are such somatic modifications as are produced as responses of the organs and tissues to stimuli, and are without direct representation in the germ plasm. In the words of Weismann: "Acquired characters are those which result from external influence upon the organism, in contrast to such as spring from the constitution of the germ."[9] They reveal an inherent power of response of the tissues and organs in a more or less definite manner according to the differentiation of the tissues and the nature of the stimulus. It may be that much of the complicated development of to-day was primarily of the nature of responses to stimuli.

The acceptance of Weismann's germ plasm theory of inheritance, strengthened as it has been by the factorial hypothesis, has for the past two or three decades concen-

[9] Professor J. A. Thomson's definition ("Heredity," 1912, p. 173) is as follows: "An acquired character, or a somatic modification, may be defined as a structural change in the body of a multicellular organism, involving a deviation from the normal, directly induced during the individual lifetime by a change in the environment or in function (use and disuse), and such that it transcends the limits of organic elasticity, and therefore persists after the factors inducing it have ceased to operate." Among his illustrations he cites: "callosities induced on the skin by pressure."

trated attention wholly on the germ plasm as the source of heredity and variation in the animal world. Ordinarily in studying the origin of characters we start with the germ and consider how factors arise and characters come to be formed from them; but there is no reason why we should not also contemplate their origin by observing their manner of appearance in the soma, and from this try to understand their transmissibility. Even if hitherto the former has alone proved fertile in results and the latter sterile it does not follow that renewed attacks on the problem with additional armament will always fail.

Callosities are a definite response of the skin to stimuli resulting from contact with some hard substance involving pressure and friction. They involve new inter-relationships of the structures concerned, and may affect the underlying tissues and even the bone on which they rest. On the factorial hypothesis the multitude of characteristics making up the complex organism are assumed to have a measure of independence; yet it is allowed that definite hereditary inter-relationships exist among them when we contemplate the body as a whole. May not some of the characteristics be directly factorial and others a result of the inter-relationships brought about in their establishment, just as in architecture certain subsidiary structural parts have to be introduced in order to admit of some major effect.[10] In any structural change, however simple, and whether germinal or somatic in origin, the complex tissue inter-relationships of the organism are involved. The old ties are disturbed and new ones are established. It is conceivable that a continuance of the application of fresh stimuli, from generation to generation, may result in a weakening of the old relation-

[10] Mr. L. Doncaster ("Heredity," Cambridge, 1911, p. 97) expresses much the same idea when he says: "The belief that 'somatic' changes could not be transmitted rests largely on the idea that every character is determined by a 'factor' or determinant in the germ-cell, but it is clear that any character is not developed directly from the germinal determinant, but by the relation existing between the determinant and its surroundings, viz., the body of the organism. If the surroundings are changed, this relationship may be altered, and the altered relation may be transmitted to the offspring, so bringing about a corresponding change in the character as it appears in the next generation."

ships and a strengthening of the new, until in the end one may supplant the other.

On a hypothetical conception of this kind it may be understood that the continued production of sternal and pubic callosities, generation after generation, has introduced such fixed and intimate inter-relationships of the structural parts concerned that in the end they come to replace the old inter-relationships altogether and with them the non-callous condition. The callosities are formed antecedent to and apart from the primary stimuli. Their appearance becomes accelerated, as it were, and they arise even before the chick is hatched and the original stimuli can be effective. They are not new characters which have come in, but are new as regards the ontogenetic time at which they appear.

The possibility of responses occurring without the original normal stimuli may be illustrated from certain of the instinctive sexual activities of the ostrich. At the breeding season the cock bird performs the sexual display known as "rolling." He crouches on the ground and with wings outspread rolls from side to side, his long neck and head also taking part, the latter striking vigorously against each side of the body alternately. Also as he approaches sexual ripeness he begins to "bromm," the sound having often been compared with the roar of a lion. The mouth being closed he inflates the esophagus until the neck as a whole becomes two or three times its usual thickness and then forcibly expels the air through the nasal passages, producing a booming noise of great carrying power, consisting of two short notes and a long one, the sequence being repeated from one to six or seven times, and serving as a guide to the farmer as to the state of sexual ripeness of the bird. Again, during actual pairing, the cock mounts upon the back of the crouching hen with his right foot upon her back and the left upon the ground, and sways the front part of the body and neck to and fro as the act is consummated.

The above are three distinctive actions on the part of the cock ostrich which are usually performed only at sexual maturity, and may be deemed to be responses asso-

ciated with stimuli from secretions or enzymes of the sexual organs. Yet occasionaly very young chicks, perhaps only a week or two old, are to be seen performing the same, though in an imperfect manner. They can "roll" almost perfectly; a chick can inflate its neck, but has insufficient strength to expel the air with enough force to produce a "bromm"; and often one chick will attempt to mount another which is resting on the ground, and begin to sway from side to side in a ridiculous fashion. May not these precocious activities be interpreted as an acceleration of responses normally due to stimuli of a sexual nature? Now they are performed wholly apart from the usual stimuli and are of no adaptive nor selection value at this early stage. They have become, as it were, so integral a part of the organism that they break out without the original stimulus; they have become transmissible. They are hardly sufficiently general to be comprised under the term "play" and, in the sense of Carl Groos, to be regarded as preparatory to the real business of life. Probably many activities of a similar precocious nature could be brought forward where an intensive study of an animal has been made. They serve to show that a physiological action is not necessarily a response to the stimuli which originally called it forth; but may appear antecedent to and independently of them.

Just as physiological activities may make a precocious or accelerated appearance so it may be that acquired, morphological characters at times appear in advance and apart from the stimuli which originally called them forth; they may become transmissible, though not germinal in the factorial sense. It is submitted that the formation of callosities, ordinarily developed as responses to pressure and friction in the life-time of the individual bird, has become thus accelerated, so that they arise at a much earlier period, even within the egg, and apart from the usual stimuli. Arising in this way a character is not germinal in the sense of having factorial representation, but is nevertheless transmissible. Though appearing before hatching it is no more germinal than it would be if developed as a definite response to the post-natal stimuli

of friction and pressure. On this interpretation a new character, to wit, a callosity, can arise either before or after hatching as a result of the responsive nature of the tissues, apart from any germinal representation.

Acquired adaptive characters, structural responses to internal or external stimuli, are by their very nature extra-germinal, and their appearance may well lead us to hesitate in accepting the germ plasm theory as a complete interpretation of everything somatic, or of everything that is transmitted from generation to generation, despite the statement by Dr. C. B. Davenport[11] that: "Upon one point all geneticists are, however, agreed . . . that we must interpret all our results in terms of genes alone."

So plastic and so responsive are the parts of the organism to stimuli that, in spite of such an embracive pronouncement, it may still constitute a subject for enquiry whether many of the adaptive relationships in organisms are not such as were originally impressed upon the individual as a result of its activities or subjection to former stimuli and which have in time become transmissible. The problem has been neglected for the past two or three decades as a result of the firm hold which the germ plasm theory of inheritance has gained over the minds of biologists and the general acceptance of the non-heritability of acquired characters. *Renewed search will probably disclose many other instances of characters appearing pre-natally which could just as well be developed as needed in the life-time of the individual, and thereby throw suspicion upon their germinal origin.* Callosities are undoubtedly the most direct and simple instances of this nature which could be adduced; we have both transmissible and non-transmissible examples in the same individual. Those whose transmissibility is established could have been formed post-natally just as readily as those produced where pressure and friction are applied to surfaces not already callous. Knowing also the responsive nature of muscles, tendons, ligaments and osteological tuberosities and the readiness with which they are modified through change of habits, it is not improbable

[11] AMERICAN NATURALIST, Vol. 50, August, 1916, p. 463.

that many now regarded as transmissible could also arise as needed as direct responses. It will certainly be legitimate to question the germinal origin of those characters whose formation can be interpreted as adaptive responses to changes to which the organism is subject.

The germ plasm theory of Weismann and the factorial hypothesis of Mendel, Bateson and others have been of inestimable value in enabling us to appreciate many of the facts of heredity. But no one imagines that they give us the completed account of evolution and adaptation, as many are beginning to feel now that their contributions can be estimated more or less in their entirety, and we get a true perspective of what they have to offer. They are and will remain important chapters in the story of variation, heredity and evolution, but they are not the whole volume; nor are they the concluding chapters, as their supporters themselves would doubtless admit. It is submitted that something is yet to be gained from consideration of how adaptive characters arise as a result of stimuli from use and disuse of parts and from environment, and how they may become transmissible, though not necessarily germinal. The germ plasm theory to a large extent and the factorial hypothesis *in toto* are sterile when we come to questions of adaptation, and natural selection has to be freely invoked, whereas practically every structure in the body bears witness to its adaptive nature.

For an acquired character to become transmissible, so that it appears independently of the stimuli which originally called it forth, is manifestly a difficult proceeding when regarded from the point of view of the hereditary structural relationships which have been established through long ages. The natural and experimental phenomena of regeneration show how deep is the tendency to maintain the established relationships of the various parts of the body. An acquired character represents some temporary disturbance of the normal relationships, but ordinarily the old correlations return with the next generation and the new are but transient, persisting for the generation only. When however these

new relationships are repeated generation after generation and maintained at their full vigor for the whole lifetime, it is conceivable that they become so impressed on the organism that they gradually overcome the old weakening relationships of parts and appear from the beginning in place of them, in other words, the character becomes transmissible, the new ties become the heritage of the organism. This, of course, is no proof of the inheritance of acquired characters, but may help us to conceive its possibility in the light of considerations engendered by the callosities in the ostrich.

The skin is more likely to show responses to environmental stimuli and to the general activities of an animal than the internal organs on account of its superficial, exposed position, and callous pads are among the simplest of structural responses and their formation is readily understood. Where temporary, as on the human hand, they are by no means likely to impress themselves permanently as new interrelationships on the surrounding parts. Where, however, as in the ostrich, they would form from the beginning and persist throughout life, from generation to generation, it is more conceivable that they would impress themselves on the constitution of the bird and their time of appearance would undergo acceleration with an independence of the primary stimulus.

The accessory, non-transmissible callosity at the ankle has not yet impressed itself so forcibly upon the general structural relationships as permanently to disturb the normal tendencies, and it has to be formed anew in each generation from direct stimuli. The hereditary median thickening is the primary one, and may well justify us in thinking that the three-toed, ancestral stage of the ostrich was of long geological duration; the new pad formed by the two-toed bird is more recent and has failed as yet to attain transmissibility. It may be that in its early days a race is more responsive to adaptive, structural changes than at a later period. In many respects the ostrich now appears senescent, and may well be expected to be less plastic than in past ages.

In general, correlated structural relationships, established through long ages, will act as a *vis inertiæ* to the introduction of acquired changes; they will represent so much heritable, inherent tendency which has to be overcome before any new relationship of parts can be established. Life-time changes of habit or of environment, as in the assumption by man of the erect habit, or the taking to water of a former terrestrial organism, are the conditions which will be conducive to acquired changes becoming transmissible, compared with those under which the responses are temporary, or continued for a few generations, or are the result of mutilation.[12] Any temporary structural relationship established, as in the decaudation experiments of Weismann and others, would manifestly be incapable of overcoming those deeper relationships which, with each new generation, find their expression in a complete tail. As Professor T. H. Morgan[13] points out, the theory of the inheritance of acquired characters "is one that has the great merit of being capable of experimental test," but he allows that "modern Lamarckians are justified in claiming that the validity of the theory can only be tested by experiments in which the organism is subjected to influences extending over a considerable period." The hypothesis here submitted is undoubtedly one which in most experimental cases would demand long period for the effectiveness of its tests.

We need not expect mutilations to become transmissible, nor most of the responses established during the life-time of an individual; but this in no way precludes the possibility for life-time responses which are continued for generations, or which may happen to strike a race at some plastic period of its existence.

[12] In the adoption of a new habit during the life-time an adaptive character may appear from generation to generation as the habit comes to be assumed, and give the appearance of being transmissible, whereas it may be formed as an ordinary response to the new stimuli. Especially where an animal is in process of changing the stimuli to which it is subject will it often be difficult to distinguish a transmissible from a responsive adaptive character which is non-transmissible.

[13] Morgan, T. H., "Evolution and Adaption," 1903, p. 230.

It is by no means anticipated that the conception of the transmissibility of characters as so many accelerated adaptive responses, involving new structural inter-relationships, and not necessarily with factorial germinal representation, will apply to all the features of an organism and serve as an explanation of the origin of heritable characters generally. Its application may be limited to such as have an adaptive significance, and can be assumed to have arisen in the first instance as a result of internal or external stimuli acting upon the soma. As will be shown in a later paper the ostrich itself, especially in the details of its degeneration, presents us with many character changes which have manifestly no adaptive significance, but are the expression of germinal changes, uninfluenced by external forces. Without question we are short-sighted in attempting to reduce the methods of evolution to some common term; as Professor H. F. Osborn points out in his new book: "The Origin and Evolution of Life,"[14] there are *centripetal* factors in organic evolution, there are *centrifugal* factors. Much of the recent work on Mendelism and mutation strongly supports the view so warmly advocated by Professor W. Bateson and Professor T. H. Morgan that germinal characters appear apart from any adaptive considerations, and the degenerative changes in the ostrich are in full accord with this; but it is by no means a complete answer to the problems of evolution, where so much appears that is directly adaptive and so little that is non-adaptive. Most genetical work during the present century has been unconnected with adaptation, yet it is one of the big problems of biology which calls for solution as insistently as ever, and it may be that a proper interpretation of the callosities in the ostrich will assist in some measure towards an understanding.

[14] Reviewed by Professor Lillie in *Science*, November 8, 1918.

# THE SELECTION OF FOOD-PLANTS BY INSECTS, WITH SPECIAL REFERENCE TO LEPIDOPTEROUS LARVÆ[1]

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THE instinctive behavior exhibited by phytophagous insects in the selection of their food-plants is always a matter of interest to entomologists, and it is one of the fundamental principles underlying the application of entomology to agriculture, horticulture and forestry. Nearly all insects show a great fixity of instinct in this respect, but a most cursory examination of the habits of almost any group will reveal a considerable variation among different species, particularly with reference to the number of plants regularly utilized as food and in the selection of closely related or of very diverse plants.

The origin and development of the association between insect species and plant host has been the basis for a considerable amount of speculation which has increased in proportion to the additional knowledge continually added through field observation, collection, and rearing of insects.

Before considering any of the theories advanced to account for the association of insects with definite plants, I shall attempt to give a very brief account of the salient facts concerning food-plants which appear to be sufficiently definite for orderly arrangement, restricting the discussion for the present, mainly to one of the better known orders of insects.

The term phytophagous with reference to insects is commonly employed in a considerably restricted and rather inaccurate sense, including only those species that feed upon the higher plants, meaning by these the ferns

[1] Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University, No. 168.

and flowering plants. Only an extremely small, almost negligible, proportion subsist upon ferns, so that from a practical standpoint, we would include only those feeding upon the Spermatophytes. This usage has developed on account of the fact that the fungi which have many insects feeding upon them, do not ordinarily engage the attention of the economic entomologist, and for convenience it is acceptable in the present connection, as very little is known concerning the specific hosts of insects living in fungi. Furthermore, the food-plant is ordinarily understood to mean the species upon which the larval or growing stages occur, for although it is common to find both the young and adult insects of the same species subsisting upon the same plant, it occurs also very frequently that the food of the larvæ and imagines of holometabolous insects is of entirely different nature. Among the many other truly phytophagous insects living in fungi are a number of families of beetles, for example, which develop in the tissues of the larger, fleshy fungi and many of these mycetophagous insects undoubtedly show a very close association with certain species of fungi. In addition, some insects subsist upon the lower fungi, yeasts and even bacteria. The biology of these latter is very imperfectly known in nearly all cases, owing to the greater difficulties attendant upon studies dealing with them. The well-known fungous-growing ants and termites and the ambrosia beetles actually cultivate certain fungi for food and other insects (undoubtedly a far larger number than is now known) subsist upon various microorganisms, although they are, to the eyes of the casual observer, feeding directly upon the substrata which really nourish the microscopic fungi, yeasts or bacteria, that in turn form the actual food for the insects. As already said, however, these symbiotic relations are in most cases only very poorly understood, and they are entirely outside the scope of the present discussion.

As distinguished from those of predatory, parasitic and saprophagous habits, the phytophagous insects rep-

resent probably nearly half of the known species, and a considerable proportion of the several orders of insects contain at least some species that are phytophagous in the sense indicated above. Some of these, like the Orthoptera, are very primitive, while others of probably equal or even greater antiquity are not phytophagous, so that it is difficult to say whether the earliest true insects were vegetarian, predatory or saprophagous.[2] This question is perhaps not a very important one, for, as will be pointed out later, a change from one type of food habits to another has actually taken place independently in several families of the highly specialized Lepidoptera.

As we might naturally expect, it is possible to point out in a very general way a progressive specialization in the selection of food-plants which parallels to some degree what appears to have been the path of evolution among insects, as determined from the criteria furnished by comparative anatomy, development and paleontology. Thus, the primitive Orthoptera appear to select their food-plants with but little discrimination, while the Lepidoptera and phytophagous Hymenoptera exhibit almost unerring accuracy in their instincts to choose certain plants and consistently to ignore all others. Beyond this, however, it is not easy to make any broad statements, for among the most highly specialized groups we find a great variability, at least in the number of food-plants admitted to the menu, as well as in regard to the botanical relationships of the plants regularly selected.

It may be argued that selection of food-plants is a somewhat dubious expression and that it may not accurately represent the condition of affairs from the standpoint of the larval insect. In most cases the larval food-plant is really chosen by the adult female, who places her eggs upon certain plants which then become of necessity the food of the resulting larvæ, which could not very readily migrate to another kind of plant even should they

[2] This last term is rather ambiguous and is rapidly becoming still more so in the light of studies recently made upon insects that subsist upon microorganisms.

be willing to do so. This objection is readily met by experimental evidence, for every entomologist is fully aware of the fact that it is ordinarily quite impossible to rear insects of restricted food habits upon other than their normal food-plants. It is true that an acceptable plant may sometimes be found by those familiar with the vagaries of related species of insects, but in such cases we may safely assume that the experimentally selected plant may later prove, in at least some cases, to be one sometimes picked out for food in nature.[3] It would be an unwarranted assumption, therefore, to suppose that the maternal instinct of oviposition does not at the present time represent fairly well the tastes of the larva. We may reasonably ask, however, whether the selection of the mother may not have impressed itself upon the larva after continual repetition or whether the taste acquired by the continual feeding of the larva may not persist into the adult, just as fondness for sweets may become a lifelong attribute in examples of the human species pampered in youth by indulgent mothers. During the progress of evolution as food-habits have become fixed, it is evident that any changing tastes on the part of the larva must have become a part of the egg-laying instincts of the mother, through the action of natural selection or otherwise, before any change of food-plants could occur. On the other hand, any change in the instincts of oviposition, not incompatible with larval tastes, might quickly become a definite characteristic of the species. If any adults should select unsuitable plants their progeny would quickly perish. The maintenance of definite preferences can thus be seen to be readily perpetuated through the action of natural selection in the survival of the fittest strains and the elimination of the unfit ones. It will be evident later, however, that subsistence on many food-plants would appear to have originated after

[3] It may also be noted that those experienced in rearing caterpillars are frequently able to rear species of unknown habits on certain plants (*e. g.*, chick-weed, *Cerastium*) on which they do not normally feed, but which are acceptable to many larvæ in the absence of their natural food-plant.

the manner of mutations, and it will, I think, be evident that we should attribute these, at least in part, to chance mutations or aberrations of instinct in the parent insects.

Before dealing specifically with the selection of food-plants, it is necessary to classify in a general way the types of food-habits generally met with in insects. Thus, Reuter applies the terms Pantophaga to omnivorous insects, Phytophaga and Sarcophaga to vegetarian and carnivorous ones respectively and Necrophaga and Coprophaga to those living upon dead animals and excrementitious material. Among the Phytophagous forms he would further distinguish monophagous and polyphagous species on the basis of the number of food-plants which they utilize. Although satisfactory so far as it goes, this fails to include several categories commonly referred to by entomologists and for the present purpose it can be readily enlarged as follows:

| | | |
|---|---|---|
| | **Pantophaga** | |
| **Phytophaga** | | **Sacrophaga** |
| **Monophaga** | | **Harpactophaga** |
| **Oligophaga** | | **Entomophaga** |
| **Polyphaga** | | |
| | **Saprophaga** | |
| | **(Partly subdivided as below)** | |
| {**Microphaga**, **Mycetophaga**} | | {**Necrophaga**, **Coprophaga**} |

In this arrangement a distinction is made between vegetarian species with a single food-plant (Monophagous), those with several definitely fixed ones (Oligophagous) and those with quite indiscriminate food-habits (Polyphagous). On the other hand predatory species (Harpactophagous) and entomophagous parasites are distinguished, as each form a very large and important group. Many necrophagous and coprophagous species really subsist on bacteria, fungi, etc., and these may perhaps be better designated as microphagous and mycetophagous.

Among phytophagous insects, the polyphagous habit is probably the most primitive and the monophagous one

the most highly specialized. It is not rare, however, to find all three types represented in otherwise very homogeneous groups. The Lepidoptera, for example, form an enormous complex of species, practically all of them phytophagous, the majority feeding upon a very restricted series of plants and representing the oligophagous habit, with a smaller series of apparently monophagous forms and a few secondarily polyphagous ones. Fortunately also, the food habits of this order as a whole, are better known than those of other insects and it can be examined with less chance of error than perhaps any other group of equal extent.

As already stated, nearly all of the larvæ of the Lepidoptera are phytophagous at the present time and there can be no question that since the order has existed this condition has prevailed. Owing to a change in the form of the trophi during metamorphosis by which the adult Lepidoptera develop haustellate or sucking mouthparts, the food of the imagines is entirely different from that of the larvæ and they subsist upon liquids, mainly the nectar of flowers.

We may then classify the food-habits of the larvæ roughly as follows:

| Food Material | Frequency of Utilization |
|---|---|
| Plant-food | Nearly all of the species |
| Bacteria | Probably none |
| Fungi | Almost none |
| Lichens | A very few, mainly in one family |
| Mosses | Almost none |
| Ferns | A very few |
| Flowering plants | Probably about 99 per cent. |
| On foliage | A large majority |
| In flowers | A few |
| In aquatic plants | A very few |
| In roots | A few |
| In tissue of herbaceous plants | A rather small number |
| In wood of shrubs and trees | A rather small number |
| In dried seeds, fruits, etc. | A very small number mainly in one group |
| Animal food | |
| On other living insects | A few isolated cases |
| On material of animal origin; wool, horn, beeswax, etc. | A very few, mainly in one group |

From this it will be seen that while the food-habits are very homogeneous, isolated cases occur where certain species have departed very strikingly from their more conservative relatives. Among these, the most interesting are those which have become carnivorous. Thus, we have in the eastern United States, a small butterfly, *Feniseca tarquinius,* which feeds upon plant lice occurring on alder. In our southwestern states there occurs also a moth of the genus *Epipyrops,* typical of the family Epipyropidæ, utterly unrelated to *Feniseca,* which feeds upon Homopterous insects of the family Fulgoridæ, and other species of *Epipyrops* are known to have quite similar habits in the orient. Also *Thalpochares,* a moth of the family Noctuidæ, is known to feed upon aphids and scale insects in Europe and Australia. Similarly the caterpillars of the Australian Cyclotorna is ectoparasitic on Homoptera of the family Jassidæ, and the larvæ of *Zaphiodiopsis* feed upon other caterpillars. A still further and more extraordinary modification is in the larva of the British butterfly, *Lycæna arion,* which is herbivorous in its early stages, but enters the nests of ants to prey upon the ant-larvæ during its final period of growth. Other scattered cases of predatory caterpillars are known, including other butterflies and moths of several families. With these the most striking feature is that the prey almost always consists of Coccids or Aphids. This association is probably due to the fact that these Homoptera are sessile or slowly moving creatures, commonly present where caterpillars occur and therefore apt to attract those of carnivorous instincts. Of interest in cennection with this, is the fact that certain phytophagous caterpillars may become temporarily carnivorous, quite regularly or under the stress of circumstances. Thus, the very abundant and destructive corn ear-worm, *Heliothis obsoleta,* commonly lays a number of eggs on the silks of a corn-ear, although nearly aways only one caterpillar finally survives in the interior of the ear where it does most of its feeding. Here the elimination

is due to a cannibalistic instinct of the caterpillar which results in the disappearance of the excess individuals, notwithstanding the fact that there is food enough for a considerable series in a single corn ear. A similar cannibalistic habit has been reported in *Hadena* and *Agrotis,* two other genera of the same family, and no less than 75 species of European Lepidopterous caterpillars are known to be occasionally predatory through temporary aberrations of their trophic instincts.

With such plasticity of behavior in several diverse families and even with *Lycæna arion* and certain small moths exhibiting a change in food habits during ontogeny, it is not difficult to regard the origin of sarcophagy in Lepidoptera as due to independent changes which have become firmly fixed in individual species or genera.

The habit of certain Tineid moths, including the clothes-moth (*Tinea*) and some of its relatives, to feed upon wool and other materials of animal origin is well known, and other non-domesticated forms of the same group exhibit similar food-habits. One African species of *Tinea* lives at the base of the horns of a large water antelope, where it forms tubes similar to those constructed by some other Microlepidoptera. The bee-moth, *Galleria mellonella,* a commensal in the hives of the honey-bee, subsists upon beeswax and bits of refuse said to contain about 20 per cent. of nitrogenous matter. Practically all of the caterpillars that subsist on foods of animal origin are more or less closely related, but not sufficiently so for us to entertain for a moment the belief that the habit has not originated independently in numerous instances. Why it should be restricted to a few groups in one part of the order, may, I think, be explained on the following basis. Among the Microlepidoptera only do we find forms able to subsist upon plant materials containing a very small amount of water (*e.g.,* seeds, dry fruits, grain, flour, etc.) as distinguished from the tissues of growing plants. Even in the wood of trees,

tunneling larvæ remain in a moist burrow where evaporation is very slow. Similarly the animal materials utilized as food are very low in water content. That we do not find Lepidopterous larvæ in moist material of animal origin is no doubt due to the fact that they do not appear to be adapted for subsistence upon the abundant microorganisms present in such materials.

Passing to a consideration of the phytophagous Lepidoptera, by far the greater part of the order remains to be dealt with. As indicated in the tabulation, practically all of these occur on the higher plants and feed almost always upon living tissue. The latter is true almost without exception of the leaf-feeding forms, although one of our common moths of the eastern states, *Pyromorpha,* is known to live upon dead and decaying fallen leaves and another of our small moths avails itself of hemlock chips. Among those which live in woody tissue, some prefer weakened or sickly trees or unhealthy branches, but almost none occur in dead wood.

Of those living on the lower plants, one small family of moths, the Lithosiidæ, subsist upon lichens and they are almost the only ones affecting these plants. This family is far from primitive, so that its association with a series of lower plants could have no significance, even if it were definitely known that the lichens are a very old group, which does not seem probable.

Mycetophagous forms of Lepidopterous caterpillars are of very unusual occurrence, in spite of the fact that several large series of beetle larvæ develop in fungi. They are found, however, and there are in North America at least two species of *Tinea* which have been bred from these plants.

In spite of the similarity of their foliage to that of the flowering plants, ferns do not commonly serve as food plants for insects. They are, in fact, strikingly immune from insect pests of all sorts. This is hardly what might be expected from the long presence of this group of plants, their enormous development in the past, and their

persistence at the present time in quite considerable abundance. Why they should be so sparingly selected as food plants does not seem to have been adequately explained.

The use of Phanerogams as food-plants is so general that it is possible to gain a much clearer insight into the conditions pertaining to them than is the case with other plants. In general the food habits of butterfly larvæ are more fully known than those of the moths, on account of the smaller number of species and the general interest taken by amateurs in this group.

An account, very complete at the time, has been given by Scudder of the food-plants of the butterflies of eastern North America.[4] A tabulation of the food-plants included in this list shows several interesting features. Fifty-five families of plants are included (not taking into account several larvæ feeding on conifers and our one predatory species) and the list contains a very representative series, drawn from both the Monocotyledons and Dicotyledons in approximate proportion to the number of species of these two sections. It is noticeable, however, that several common families, the Iridaceæ, Orchidaceæ, Caryophyllaceæ, Euphorbiaceæ, Vitaceæ, Primulaceæ and Rubiaceæ are entirely omitted, that only one species occurs in the Labiatæ, or on the Umbelliferæ, and that only a very few affect Compositæ. We may readily see that the generally strong-scented Labiatæ and Umbelliferæ and the milky Euphorbiaceæ might require great adaptation on the part of larvæ eating them,[5] but the omission of the other families if not entirely a matter of chance must rest upon some less evident basis than the foregoing. Among the other plant families the number of species of caterpillars compared to the number of eastern American genera, included in each family that is

[4] This list has been used as a whole as it is complete in itself, and to attempt to add to it and emend it by the present writer, from more recent literature, would improve it but little for the present purpose.

[5] This is true only of the butterflies in this list; many other leaf-eating larvæ feed in abundance on these plants.

fed upon, varies exceedingly, from 1:100 to 1:1 or even 1.6:1 in the case of the Rutaceæ. The average is about 1:4, but there is no tendency for the ratios to fall near the mean and their distribution if not a matter of chance, must have been determined in relation to their environment, no doubt to a great extent by their struggle for existence with other plant-eating forms.

If we examine the food-plants of the genera or higher groups of butterflies, we find that most of them exhibit well-marked preference for certain, usually related plants. The food-plants of the British butterflies are unusually well known, and Tutt has recently given in his work on "British Butterflies" a digest of their preferences which he finds to be closely similar to those of the nearctic forms previously considered by Scudder. Gathering his more definite data together and using his terminology for the groups, the food-habits may be tabulated as follows:

| | |
|---|---|
| Vanessids | Urticaceæ Compositæ, etc. |
| Argynnids | Violaceæ exclusively |
| Brenthids | Violaceæ generally |
| Melitæids | Various plants |
| Pierids | Cruciferæ essentially |
| Anthocarids | Cruciferæ essentially |
| Limenitids | Various plants |
| Apaturids | Various plants |
| Satyrids | Gramineæ almost entirely |
| Coliads | Leguminosæ almost entirely |
| Gonepterygids | Rhamnaceæ |
| Ruralids | Various plants (more or less fixed in some subdivisions) |
| Lycænids | Diverse food-habits |
| Chrysophanids | Polygonaceæ almost exclusively |
| Urbicolids | Various plants |
| Sub-groups Thymelicines | Gramineæ mainly |
| Urbicolines | Gramineæ mainly |
| Cyclopidines | Gramineæ mainly |
| Hesperiines | Leguminosæ and various other plants |
| Papilionids | Various plants; several groups with particular food-plants |

Viewed in this way, it is seen that barring many exceptions, there is a general tendency, much more pronounced

in some groups than in others, to select plants of very specific families or even genera. This must not be understood to mean that the individual species of insects affect indiscriminately many or all members of the plant group, but that their normal food-plant or plants do not fall outside the group. With the exceptions in mind, the fixity of the instinct to feed on only certain kinds of plants is all the more extraordinary, for we cannot readily dismiss it as a physiological or nutritional necessity.

An interesting light upon the effect of the environment in influencing the selection of food-plants, is furnished by several widely distributed species and genera of butterflies. Thus several species of *Vanessa* have quite identical food-habits throughout the entire holarctic region and the same is true of several very closely allied palæarctic and nearctic species belonging to the same group, while, as mentioned above, the general food habits of the larger groups run closely parallel among their representatives on the two continents. Still more interesting in this respect are the butterflies of the closely related genera *Catopsilia* and *Callidryas* which restrict themselves to the Leguminous genus *Cassia*. These butterflies occur in the nearctic, neotropical, Indo-Malayan and Australian regions and such species as have been reared show this preference, which is probably universal. The well-known genus *Papilio* supplies some similar peculiarities in that several world-wide groups of the genus are restricted to certain closely related groups of plants (*e. g., Aristolochia, Citrus,* etc.). On the other hand, one North American species, the common *Papilio glaucus,* is known to affect food-plants belonging to no less than fifteen different families of plants. With such constancy in the most remote quarters of the globe among related species of this genus and with one species in a single region regularly developing on the most diversal plants, we must believe that the fixed instincts of some species are not to be led astray by the many temptations offered even by the varied plants of widely separated zoological

regions, while those of other species are so loose that they restrict their owners only to a comparatively very small extent. Such conditions certainly point to instinct as the determining cause of food selection, rather than physiological adaptation to specific kinds of plants.

In connection with cosmopolitan butterflies, Scudder noted many years ago, that there are no species of remarkable distribution known to feed upon Leguminosæ or grasses, although these plants are cosmopolitan and harbor many species. I am inclined to believe, however, that this has no significance, particularly in view of the aforementioned Coliads that feed upon *Cassia* in various parts of the world.

Many other groups of Lepidoptera conform quite closely in food-habits to the butterflies, although some show greater diversity, especially in affecting different parts of the plant, and it may be said in general that the larger moths are less apt to be monophagous than the butterflies.

Among the hawk-moths or sphinx-caterpillars several subfamilies show a restriction to groups of related plants, while others do not. Thus in this cosmopolitan family, one subfamily (Chærocampinæ) feeds on Vitaceæ, with an admixture of diverse other plants, another (Macroglossinæ) on Caprifoliaceæ exclusively, another (Sphingineæ) to a great extent on Oleaceæ together with other plants as different as Conifers, Solanaceæ, Euphorbiaceæ and Labiatæ, while one (Smerinthinæ) exhibits no apparent preference.

The family, Aegeriidæ, or clear-winged moths, live in the larval stage in the interior of plants, tunneling through the tissue. They affect a very wide series of plants, herbs, shrubs and trees, as can be seen from the following abbreviated list which represents the range in habits of some of our eastern North American representatives; stems of Cucurbits, *Vernonia,* blackberries, currant, grape; wood of pine, willow, lilac, maple, oak, peach; roots of *Clematis*, persimmon, blackberry, *Eupa-*

*torium;* stumps of oaks cut the previous year; and oak-galls. Notwithstanding such diversity within the family, the individual species are nearly monophagous, or oligophagous on related plants. Such a condition would seem to have arisen through sudden mutations in instinct rather than from numerous smaller variations having a selective value, for in the latter case we should find polyphagous forms developing in some places at least.

Another, much smaller family of rather generalized structure, the wood-boring Cossidæ, have habits similar to many of the Sesiidæ, but their selection of food-plants is very different. Typically they are oligophagous, but some species, including the well-known and destructive shade-tree pest known as the leopard-moth (*Zeuzera pyrina*) introduced from Europe into the eastern United States, has been bred in this country from an almost endless variety of shrubs and trees, as it has been in Europe also. As listed by Chapman, the American food-plants belong to twenty-two families of plants and to nearly fifty genera. Almost all that can be said of the leopard-moth's bill-of-fare is that it includes no conifers.

*Cossus ligniperda,* another European species is strongly polyphagous, but many of the exotic species appear to avail themselves of a rather restricted diet. As this latter is perhaps due to lack of knowledge, it may be unwise to draw any conclusions at present.

In other families of moths the same phenomenon is frequently encountered. The large group of Noctuidæ, comprising the owlet-moths, feed mainly upon the foliage of a wide rang of plants, while the list of food-plants for the numerous species varies greatly in extent. To attempt to classify the food-habits of this group would require much time and space, but it may be said that there are species in certain genera, as, for example, the cotton boll-worm, which appear to have rather suddenly enlarged their range of food-plants as compared with that of related species of the same genera.

In regard to the uniformity of food-plants during on-

togeny, the statement has been made that some Lepidopterous caterpillars occur on a greater range of plants when young, or at least that they will readily feed upon certain kinds during the earlier instars, and refuse them later, so that their diet becomes more restricted as growth progresses. This statement has been in turn used as an argument that oligophagous forms are derived from more restricted feeders, and that they repeat in a way their history by the limitation of their food-plants during successive instars.

Some elaborate experiments on the feeding habits of the gipsy-moth reported on by Mosher tend to discredit this supposition, however. As is well known, the gipsy-moth occurs on a wide range of plants, but shows well-marked preferences for certain among them which represent its favored food. These experiments were carried out in the extensive detail possible only when dealing with insects of great economic importance, and although planned for another purpose, furnish valuable data upon this point. It appears that on a number of their numerous food-plants, the gipsy-moth caterpillars show an inability or at least an unwillingness to feed either during the very early or during the later larval stages. On some kinds of trees the early larvæ failed to develop and on others the latter stages did not feed, although the young ones did so. This diversity of behavior is in part due to the fact that young larvæ cannot usually feed upon conifers, while the older ones eat the foliage of these trees voraciously; but it is by no means due to this alone, so that we can say that the juvenile preferences of the larvæ become transformed or changed as growth progressed. With an active polyphagous caterpillar like the gipsy-moth in which the larvæ often migrate to other species of plants during growth, it is possible for such changes in diet to take place regularly in nature, although such could not ordinarily occur with oligophagous species without tending greatly to reduce the chances of the species to survive. As the necessity for migration is most acute in the case of very abundant species, they are open to more

temptations to avail themselves of a variety of foods and we find that it is usually the most abundant species of any group that are polyphagous. Conversely we may say also that polyphagy, when present, greatly increases the chances for the larvæ to secure the necessary amount of food for complete growth and tends to cause the species to become excessively abundant. Once under headway, these two processes will act together and result in the production of dominant species that tower above their fellows. Examples of this are seen in the gipsy-moth,[6] the *Cecropia*-moth, the army-worm, *Papilio glaucus* mentioned above, the woolly bear (*Isia isabella*), etc. We must not lose sight of the fact, however, that this is only one of many factors influencing dominance. The milkweed-butterfly, one of our most abundant native species develops on a very common plant (*Asclepias* almost exclusively) and is thought also to be a protected species.[7] Its dominance may be interpreted, like that of many destructive agricultural pests, as due to a plentiful and unfailing food supply, coupled with other pre-requisites inherent in the insect itself.

In spite of the many exceptions and variations which have been enumerated, the fact stands out clearly that the Lepidopterous insects show a very fixed instinct to select definite plants for larval food; that many are extremely precise in this respect, some less so, and others quite catholic in their tastes. Furthermore there is much to show the existence of a so-called "botanical instinct" in species, genera and even families, whereby evidently related plants and these only serve as food. A few species have departed from the general habit so far that they have become carnivorous, and among the others we find every gradation between the extremes of monophagy and polyphagy.

It has been claimed that the food habits may be modified experimentally, in that caterpillars reared on a

[6] This is true also in the native habitat of this species, aside from the decimation by parasites which occurs more abundantly in Europe.

[7] *i. e.*, distasteful to its enemies and exhibiting warning coloration.

strange plant (where they could be induced to select it) give rise to moths whose progeny more readily accept the new plant. It is very difficult to accept such evidence, at least as having any general application, without very clear and incontrovertible proof. If such transformations can occur so easily and become hereditary so quickly they should have entirely destroyed the coherent habits now existent, during the enormous period which has elapsed, for example, since the violent-feeding Argynnids were differentiated, since the holarctic and nearctic Vanessids have been separated, or while the world-wide Aristolochia-feeding Papilios were attaining their present distribution. That such a change has actually occurred in the case of other groups seems equally evident, although, as has been shown, we can more easily believe that they may have arisen through mutations in maternal instinct not incompatible with larval tastes and then only in extremely rare cases and confined to certain groups.

With a knowledge of the specificity of proteins in different living organisms and their apparent differentiation as a replica of the genealogical history of the animal and plant kingdoms, has come the suggestion that the dependence of monophagous or oligophagous insects upon specific plants rests upon a physiological basis, and that particular proteins or vitamines are an actual necessity for growth and development. A survey of the field does not seem to bear out this supposition, however plausible it may appear at first sight. With monophagous larvæ, it will serve as a reasonable explanation, and with oligophagous ones also so far as the individual species are concerned, especially where such species select a series of related plants. With those that select only a few plants, however, and at the same time such as are evidently not closely related, it does not seem so appropriate. It is when we compare the lists of food-plants of several oligophagous species that it appears to fail completely to meet the requirements. Thus we find, referring again to our North American butterflies, such combina-

tions as Leguminosæ with Pinaceæ, or with Rhamnaceæ, Polygonaceæ, Cupuliferæ in the case of different larvæ. More uniformity should certainly be expected if the selection of diverse plants depended upon the actual chemical characteristics of the plant tissue. We should have also to assume that the digestive functions of the cecropia caterpillar with its sixty-odd food-plants were fundamentally different from those of monophagous caterpillars.

There is much in the behavior of certain species to suggest that food-plants are selected on the basis of odor by the parent female and also accepted on the same basis by the larvæ. Experiments with cabbage butterflies by Verschaffelt and others show that these insects are attracted by the mustard oils present in these plants, and it has also been shown that caterpillars will feed on other plants which have been treated with one of these oils. Similar behavior in the most diverse insects is also known in the attraction exercised by specific fermentation products (*e.g.*, to *Stomoxys, Drosphila,* etc.). The distaste of mosquitoes for oil of *Citronella* is well known, as is also the attractiveness of this same substance for fruit-flies of the genus *Dacus.* That the same cabbage butterflies have definite dislikes in the way of plant odors has recently been claimed by the Russian entomologist Schreiber, who found that *Pieris brassicæ* would not attack cabbages planted in close proximity to tomatoes. *Pieris rapæ* does not seem to behave similarly, however, and this dislike is probably not general among the crucifer-eating Pierids.

Very recently McIndoo has published some observations showing that caterpillars readily react to the odors of several essential oils and to those of various plants. This, taken together with the fact that *Pieris* will feed upon strange plants treated with mustard oil, would suggest that odor is an important factor in the selection of food-plants. Queerly enough, however, he found that the response to their own food-plants was no more rapid than to the other substances, and even slower in some

cases. As the smearing of the oils of one plant on another does not occur in nature, the important point to discover is whether there is really any similarity of odor in the several plants of diverse groups that are sometimes utilized by a single species of insect. The facts alluded to above in regard to the wide variety of selections made by different species would seem to answer this question in the negative, as would our own human sense of odor, which latter may, of course, not be reliable when dealing with a group of animals so different from man. We may, I think, rest assured that odor frequently guides the insects to their food-plant, but we can not believe that oligophagous or polyphagous species have become accustomed to a variety of plants due to a confusion of similar odors. There does seem, however, to be one very striking exception to this among the Pierid butterflies. As said before, these butterflies are confined to Cruciferæ almost exclusively, but one of our species not infrequently occurs on the garden "nasturtium" (*Tropæolum*). That the pungent taste of this plant is much like that of a Crucifer is well known and further attested by the common name, as the true nasturtium of the botanists is a genus of Cruciferæ, while the garden nasturtium is a Geraniaceous plant.

On account of the very close biological association between insects and plants in many ways it is true that the two have been mutally specialized until they have become highly modified in reference to one another, but this is not the case with food-plants, as no benefit ordinarily accrues to the plants and any idea of parallel evolution must be restricted to a development of undesirable attributes on the part of the plants and adaptations on the part of the insects to overcome such barriers to feeding.

To avoid these numerous difficulties, it seems clear that the selection of food-plants by the Lepidopterous insects so far mentioned, must be considered as dependent upon one or several of a number of factors. Among these we must include the following:

1. The odor of the plant, and also its taste, which is no doubt closely connected with odor. Associations reasonably placed in this category would be the oligophagous species occurring, for example, on various Cruciferæ, various Umbelliferæ, and various Compositæ. An additional argument for the importance of this factor is seen in the less common utilization by the same insect of several plants in a family like the Solanaceæ[1] where a more or less similar odor does not become a family characteristic.

2. Some attribute of the plant, perhaps an odor but far less pronounced to our own senses than those mentioned above. Species restricted to plants like Leguminosæ or Violaceæ may be considered in this category. Undoubtedly there is some attribute of such plants which insects can recognize in a general way and not as a specific characteristic of some single plant species or genus. The "botanical instinct" of some caterpillars that has frequently been commented upon would appear to be an exaggerated power of recognition of this sort.

3. A similarity in the immediate environment or general form of the food-plant. The effect of something of this sort is seen particularly in oligophagous and also polyphagous caterpillars feeding mainly on trees or shrubs, such as the gipsy-moth, *Cecropia* moth, etc., and those of certain species like some of the Arctiid moths that feed upon a great variety of low plants.

4. Apparently chance associations that have become fixed, whereby diverse plants are utilized by oligophagous species. Secondarily polyphagous species show these in an exaggerated form. On account of their comparatively rare occurrence these seem to be analogous to structural mutations, although they appear to be strictly modifications of instinct. As has been pointed out on a previous page, these are much more apt to occur in some groups (families and genera) than in others.

[8] Possibly in this family, however, the matter may rest upon a physiological basis, on account of the common occurrence of powerful alkaloids in these plants.

# THE MANIPULATION AND IDENTIFICATION OF THE FREE-SWIMMING MASTIGOPHORA OF FRESH WATERS

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Not the least among the problems that confront the worker in systematic protozoology is the identification of the minute, free-swimming Mastigophora, representatives of which occur in all cultures, often in great numbers both of individuals and of larger groups. The writer's attempts to find some means of making their identification easier and more certain have resulted in the working out of several methods of dealing with these elusive forms, methods which have been used with success in the laboratory examination of material. They are here presented with the hope that they may be a similar assistance to others.

For the purposes of identification it has been found helpful arbitrarily to divide the Mastigophora into two great groups, on the basis of size; placing those the majority of whose species measure about 12 microns or less along the antero-posterior axis in one group, and those of greater magnitude in the other. It is among the species in the first group that the difficulties in identification seem usually to occur, and it is with this division, therefore, that this paper deals.

The genera included within this first arbitrary division fall within the first four orders of the mastigophora, thus:

SUBPHYLUM MASTIGOPHORA

Order Monadida

Genera: *Mastigamoeba*
*Cercobodo*
*Cercomonas*
*Physomonas*

ORDER HETEROMASTIGIDA

Genera: *Elvirea*
*Dinomonas*
*Pleuromonas*
*Spiromonas*

ORDER PHYTOMASTIGIDA

Genera: *Amphimonas*
*Hexamita*

ORDER EUGLENIDA

Genera: *Cryptoglena*
*Notosolenus*

Almost all the mastigophora, but more especially the minute forms, vary in size and shape within the limits of the species. Individuals of apparently the same species are often found half, and even a fourth, of the size which is normal for that species, while abnormally large forms are more rarely encountered. It is the smaller forms which are the most puzzling. These are probably due to: (1) the division of the adults, for binary fission along the line of the antero-posterior axis is the prevailing mode of reproduction among the Mastigophora. Where division is occurring rapidly, in a crowded culture many such forms will be found. Often the young again subdivide, even before reaching adult proportions. This gives rise to individuals of a species varying widely in size, (2) to the fact that the adults themselves vary in size, even where division is not rapidly taking place, and (3) to the possible production in some forms of swarm spores. Possibly when the life histories of a greater number of the smaller flagellates become known, it will be found that many species multiply not only by binary fission, but by multiple fission as well, either within or without the cyst. It is not unusual to find individuals of a colonial form which have freed themselves, or have been broken away, from the parent community, and which are, temporarily, at least, living an independent

existence. Adventitious individuals may be derived from such colonial mastigophora as *Spongomonas, Anthophysa, Dendromonas, Uroglena,* and *Ramosonema.*

The following key, which was devised by the writer, has been found helpful in the identification of the minute forms, and in fixing in mind the characters that are the most prominent, and that are useful, under the microscope, in identification. The key includes practically all the forms of the minute mastigophora that are likely to be found in the waters of bogs, ponds, roadside ditches, creeks, and brooks, and putrefying infusions, in so far as these have been accorded systematic place. These forms occur over and over again, and their identification has been found to be a matter of less difficulty than was at first thought, before a definite plan for handling them was formulated.

Key to the Minute Mastigophora, Those Roughly About or Below 12 Microns

*A*. Antero-posterior diameter normally about, or less than 8 microns.
- *B*. Flagella 2, body kidney-shaped, the smallest of the mastigophora ........ *Pleuromonas*
- *BB*. Flagella 3, two trailing, one extended forward ..... *Elvirea*

*AA*. Antero-posterior diameter normally greater than 8 microns.
- *B*. With one flagellum.
  - *C*. With greenish chromatophores ........ *Cryptoglena*
  - *CC*. Without chromatophores.
    - *D*. Flagellum stiff except at tip ........ *Notosolenus*
    - *DD*. Flagellum not stiff, pseudopodia present .... *Mastigamoeba*
- *BB*. With more than one flagellum.
  - *C*. With 2 flagella.
    - *D*. With one flagellum at each extremity of the body ........ *Cercomonas*
    - *DD*. Both flagella at same extremity of body.
      - *E*. Body ovate, the anterior extremity the smaller ........ *Dinomonas*
      - *EE*. Body not normally ovate.
        - *F*. Body spherical ........ *Amphimonas*
        - *FF*. Body not spherical.
          - *G*. Body ribbon-like, twisted .......... *Spiromonas*
          - *GG*. Body not twisted.

*H.* Body drawn out bluntly posteriorly, obliquely truncated anteriorly .................... *Physomonas*

*HH.* Body drawn out into an acuminate tip posteriorly, not truncated anteriorly ............ *Cercobodo*

*CC.* Flagella more than 2.

*D.* Flagella 3, two trailing, one extended forward .. *Elvirea*[1]

*DD.* Flagella four, anteriorly extended; posterior produced into 2 filamentous appendages.... *Hexamita*

In dealing with these minute, free-swimming forms, it was of first necessity to devise some means of keeping them quiet and within the field of the microscope when the higher powers were in use. Several methods both of retarding movement and of killing were used, but those which gave the best results were the following.

For the first examination of any sample, a small drop of the culture was taken and mixed on a slide with a drop of very viscous gelatine solution,[2] and the whole thoroughly stirred together. Or often several drops of the culture were mixed with an equal part of the gelatine solution in a watch crystal and used on the slide when needed. Such a preparation would not keep the protozoa confined within it alive for more than half an hour, however, due to the occlusion of the necessary oxygen.

The drop on the slide was now carefully flattened out and examined without a cover glass under a low power (16 mm. objective and 10x eyepiece) to ascertain whether the solution were of a viscosity great enough to check sufficiently the movements of the flagellates. If not, it was allowed to concentrate still more by evaporation until properly viscous, and then covered with a cover glass. Magnification with the 4 mm. objective and the 8x and 10x eyepieces was found to be great enough for the identification of most of the forms.

The gelatine used in this method must be of the best grade and perfectly fresh and clear. It may be slightly

[1] *Elvirea*, because of its variability, is placed both here and in division *AA* of the key.

[2] See formulary of reagents at end of paper.

agitated before adding to the culture drop. This includes numerous minute air bubbles, around which the animals may gather and so become concentrated, if the solution is at first thin enough to allow them to make their way through it. It was found that gelatine that had stood ready made up for some time in a warm room became cloudy in appearance and stringy in texture, due to the growth of mould plants and colonies of bacteria.

Another method of retarding the motions of the flagellates, which was partially successful with such minute forms as *Pleuromonas jaculans* and *Elvirea cionae,* was to chill the slide and its water drop thoroughly on a block of ice. This was tried in midsummer, when the sudden reduction in temperature of water that had been quite warm (the culture having stood in the sun) apparently paralyzed the organisms, but they regained their wonted activity after a few minutes' time, since the slide could not very conveniently be kept chilled under the microscope.

The favorite method of the writer for quieting without killing was to utilize a minute aquarium, of a sort that might be used even under the high powers. This was constructed by cutting out a circle of very thin typewriter manifolding paper of good grade and firm texture of slightly less diameter than that of the cover glass, and then cutting from the center of this a concentric aperture about 5 mm. in diameter. This was affixed to the slide with a ring of thin balsam or castor oil, applied with a fine camel's hair brush; the water drop placed in the center, and the cover glass, also ringed with thin balsam or oil, carefully lowered thereon. The oil or balsam sealed the cover glass, and the paper kept it from descending far enough to crush the incarcerated organisms.

At first the flagellates in such an aquarium swim about at their normal rates of speed, but after a time they become stupefied, probably because of the gradual exhaustion of the oxygen supply, and their movements become progressively slower, until finally they cease altogether.

The flagella continue to beat for some little time after the animal has come to rest.

This offers a good opportunity for the observation of the natatory habits, and, as the animals quiet down, for making a closer examination for identification, using higher powers. The smaller the water volume in such a micro-aquarium, the sooner the stupefaction of the confined organisms takes place. The forms studied in the micro-aquarium were usually stained *intra vitam* before their incarceration.

The stains most frequently used for *intra vitam* work were methylene blue (not methyl blue) and methyl green.[3] With each of these a saturated aqueous solution was prepared and diluted to the desired strength.

The stains which were used for killed specimens were methylene blue (not methyl blue), methyl green, gentian violet, and safranin.[4]

The examination of the imprisoned *intra vitam* stained animals has the advantage over the killed and stained ones that it shows the position and action of the flagella, and so leads to a more correct idea of how this should be represented in a drawing. Occasionally one meets with the drawing of a form in which the flagellum is represented as thrown into graceful undulations, whereas, in life, it may be only the tip of that organ that is motile. It was found that frequently the killing reagents caused the flagella to assume unnatural attitudes.

In both the killed and the *intra vitam*-stained animals the flagella takes the stain least of all, often appearing but very faintly, if at all, colored. In *intra vitam* staining, care must be taken not to kill the creatures with too much stain in the attempt to make the flagella stain more deeply.

Killing, in order that examination might be made with the 1.8 mm. objective, using oil, was accomplished by the use of the various well known reagents, such as tannic

[3] See formulary of reagents at end of paper.

[4] See formulary of reagents at end of paper.

acid, osmic acid, acetic acid, formaldehyde, and mercuric chloride solutions.[4] The best results, however, were obtained by the use of a 1 per cent. aqueous solution of copper sulphate, a reagent which was hit upon in the attempt to find a medium in which death occurred with a minimum of distortion of the flagella. In nearly all cases the animals very gradually subsided into immobility without any distortion whatever. A .5 per cent. solution kills them much more gradually. These solutions must be made up with accuracy, and may be most delicately prepared by counting drops of water and of concentrated copper sulphate solution, as they come from the tip of a finely drawn out pipette. One hundred drops of water from a pipette the diameter of whose tip measures about 2 mm. makes a sufficient quantity to last for months.

The killing and staining was accomplished in either of two ways, either by killing first and staining afterwards, in which case any of the killing reagents given at the end of the paper was used, followed by the stain, or by performing both operations simultaneously. This may be done by using strong stains. The material can be stained either on a slide, or in larger quantities in a watch crystal. Where the animals were extremely abundant, as they usually were in surface scums or decaying cultures, the latter method was found to be the best, for with the larger quantity of water both the killing reagent and the stain could be more delicately controlled. Several watch crystals were placed side by side and various gradations of color secured.

The killed specimens were examined at once, and fresh preparations frequently made. Complete disintegration of these tiny forms takes place a short time after they have been killed. This is preceded by a distortion of the body.

On the whole, *intra vitam* staining, with the animals retarded in the gelatine solution, or stupefied in the micro-aquarium, gave the best results. With animals treated thus, the magnifications afforded by the 8x and

10x eyepieces and the 4 mm. objective were found great enough for the majority of the forms.

In the following descriptions of the genera and species the attempt has been made to indicate the characters which are the most prominent ones of the members of each group, those by which the identification can usually be made. Hitherto less attention than the subject seems to deserve has been given to the manner in which these lower forms make their way through the water—to what may be called their natatory habits. Many forms show natatory habits which seem to be of a constancy and a distinctness sufficient to warrant their use as characteristics for identification. This feature has been given attention in the following descriptions because it has been found a helpful one in identifying the forms.

It is here suggested that the vibratory motions which some of the smaller flagellates, like *Pleuromonas* and *Elvirea,* exhibit, may be due in part to the influence of pedesis, or Brownian movements. Carpenter states[5] that all particles suspended in water below $1/500$ of an inch (49 microns) exhibits this phenomenon, and it is a matter of observation that the smaller the particle the more pronounced the vibration.

### ORDER MONADIDA

GENUS MASTIGAMOEBA Schultze.

Body amœboid, changing shape slowly as pseudopodia are protruded, usually from the sides or posterior portion of the body. Flagellum long, fine, and not easily seen at first in its entirety, because of its rapid motion. These forms move with either a true amœboid motion, or swim by means of the flagellum. Conn states that in swimming the pseudopodia are retracted, yet we have noticed frequent exceptions in both species. The swimming of these species is clumsy, due perhaps to the irregularity of the body.

[5] "The Microscope and Its Revelations."

FIG. 1. *Mastigamoeba longifilum* Stokes (10–15 μ), 2 individuals.

FIG. 2. *Mastigamoeba repetans* Stokes (8–10 μ), 2 individuals.

FIG. 3. *Cercobodo mutabilis* Kent (8–12 μ), 3 individuals, 1 in the pseudopodia-bearing stage.

FIG. 4. *Cercomonas longicauda* Dujardin (10–13 μ), 3 individuals, 1 protruding pseudopodia at the posterior extremity of the body.

FIG. 5. *Cercomonas crassicauda* Dujardin (6–9 μ), 1 individual of typical form.

FIG. 6. *Physomonas elongata* Stokes (10–13 μ), 3 individuals.

FIG. 7. *Elvirea cionae* Parona (5–8 μ), 2 individuals.

FIG. 8. *Dinomonas vorax* Kent (8–15 μ), 3 individuals.

*M. longifilum* (Fig. 1) seems to be the most common species in the waters about Ithaca, N. Y., and New Haven, Conn., though *M. repetans* (Fig. 2) was often seen. The former is the larger, the more hyaline, and furthermore possesses at least one quite prominent contractile vacuole. No difference in the length of the flagellum in the two species could be observed.

GENUS CERCOBODO Kraasilstschik.

Body changeable in form from almost globular to elongate, with the posterior extremity usually more or less drawn out, frequently acuminate. This latter form is the one under which the species most frequently appears. The two flagella arise from the anterior end of the body. Often an amœboid form of body is assumed, and locomotion is effected by blunt pseudopodia.

Because of its instability of form, the one species which is the most common has been relegated successively to the genera *Dimastigamœba, Dimorpha,* and *Cercobodo.* The single contractile vacuole is usually present and prominent. The one species observed seems to conform to the *Cercobodo mutabilis* of Stokes (Fig. 3).

GENUS CERCOMONAS Dujardin.

Body globular to ovate, pointed at the anterior and posterior extremities, from each of which arises a long flagellum, the posterior of these being the stouter, a trifle the shorter and less motile. The pseudopodia, which are occasionally produced, are not as well defined as those in the two preceding genera, and are limited to the posterior fourth of the body. These were not frequently seen.

Two species are fairly common: *C. longicauda* (Fig. 4), and *C. crassicauda* (Fig. 5). They may be distinguished by their difference in size.

GENUS PHYSOMONAS Kent.

Body changeable in form, though not possessing pseudopodia, and varying from elongately ovoid to ovoid

pointed at the posterior extremity. The anterior portion of the body is normally obliquely truncated, from which arise two flagella of unequal length.

*P. elongata* (Fig. 6) is fairly common in all waters of ponds and bogs, particularly among sphagnum, though it seems never to occur in crowded cultures.

ORDER HETEROMASTIGIDA

Genus Elvirea Parona.

The body is pear-shaped, and though it may elongate and contract slightly during swimming, it is quite stable in form. Of the three flagella which arise from the anterior extremity, the stouter, shorter one vibrates forward, and is the organ of locomotion. The other two trail behind.

*E. cionæ* (Fig. 7) is apparently not a very common species. It was found sparsely in the clear cold waters of springs and creeks.

Genus Dinomonas Kent.

Members of this genus resemble those of the preceding one in the general shape of the body, but they are larger and possess but two flagella, both of which extend forward from the more acute anterior extremity. The contractile vacuole is usually clearly visible and located in the rounded posterior region.

Fig. 8 was found abundantly in the scums of various infusions of grasses and leaves, and conforms to the *D. vorax* of Kent. *D. tuberculata* (Fig. 9) was often found in the same infusions with *D. vorax*. It is possible that this may be merely another form of the latter species.

Genus Pleuromonas Perty.

Body either kidney-shaped or ovate, the two long flagella arising from a depression in the venter which is not, however, invariably present.

*P. jaculans* (Fig. 10) is often very common in stagnant waters wherever there is decomposing vegetable matter

present. Some infusions will be colored a milky hue from the multitudes of these forms within them. The individuals of the species vary considerably in size, some being no greater than 2 microns along the anterio-posterior axis. The majority of the individuals, however, lie within the limits of 3 to 8 microns. They may be recognized at once by their peculiar agitation and habit of leaping or jerking about from place to place. Individuals may sometimes be discovered lying quiet, except for a gentle vibration—a motion which, it has been suggested earlier in this paper, may be due to the influence of pedesis. These quiet forms are to be found usually near the edges of masses of disintegrating material, where they are likely to be overlooked. The bodies are often particularly hyaline, and the flagella difficult to make out. Iodine (an alcoholic solution, with potassium iodide, as given under No. 2, Section B of the Formulary of Reagents at the end of the paper) as a stain after killing was found to be the best for these minute forms. In some cases the flagella could be made to take the stain well, in others not. Those killed with the stain itself seemed to be better colored than those stained after having been killed with some other reagent.

Genus Spiromonas Perty.

Body leaf or ribbon-like, either flattened or more commonly twisted spirally, with one or two turns, very variable. Kent says that these forms may even assume an amœboid form of body. Of the two flagella that arise from the anterior tip of the body, one trails, one extends forward and vibrates with great rapidity.

The variable *S. angustata* (Fig. 11) seems to be quite common in infusions of all kinds, but particularly abundant in those of hay, swamp grasses, and the like.

### ORDER PHYTOMASTIGIDA

Genus Amphimonas Dujardin.

Body globular, either attached by a fine posterior pedicel, or free-swimming—the latter being apparently the

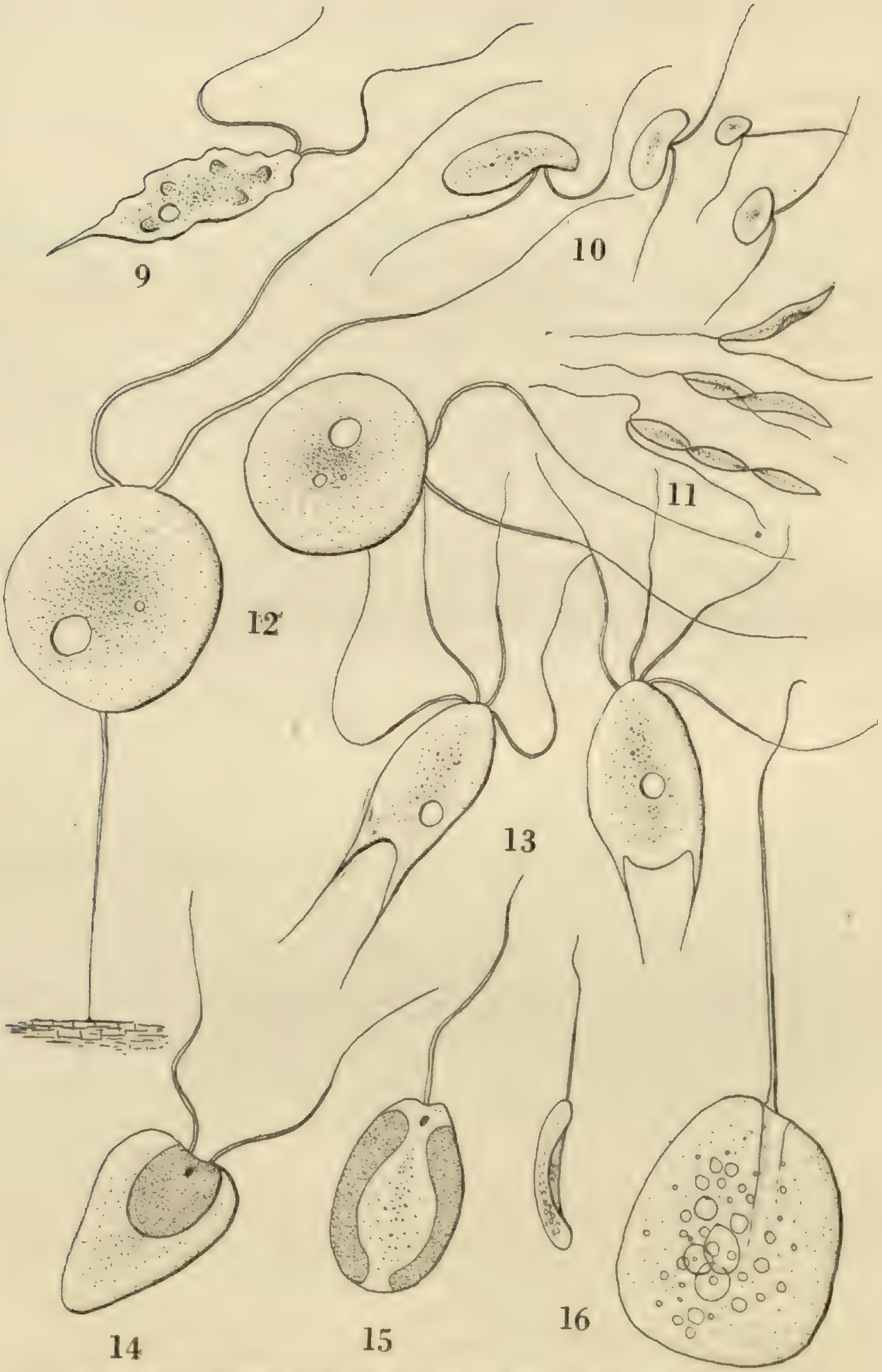


FIG. 9. *Dinomonas tuberculata* Kent (8–15 μ), 1 individual.
FIG. 10. *Pleuromonas jaculans* Perty (3–9 μ), 4 individuals.
FIG. 11. *Spiromonas angustata* Dujardin (8–10 μ), 3 individuals.
FIG. 12. *Amphimonas globosa* Kent (10–12 μ), 2 individuals, 1 free, 1 attached by a posterior pedicel.
FIG. 13. *Hexamita inflata* Dujardin (10–12 μ), 2 individuals.
FIG. 14. *Cryptoglena conica* Ehrenberg (10–12 μ), 1 individual.
FIG. 15. *Cryptoglena pigra* Ehrenberg (10–12 μ), 1 individual.
FIG. 16. *Notosolenus orbicularis* Stokes (7(?)–15 μ), 2 individuals, 1 turned sidewise to show the concavo-convex shape of the body. This latter form may be, perhaps, another species.

more common condition—by means of two long, fine, equal, anterior, rapidly vibrating flagella. In the free-swimming stage the posterior pedicel is usually absent, though occasionally individuals may be seen trailing after them pedicels of different lengths. These may be pedicels in various stages of retraction into the body.

*A. globosa* (Fig. 12) seems to be of rather rare occurrence in the waters of ponds and ditches among decaying aquatics.

GENUS HEXAMITA Dujardin.

Body very changeable in form, with two long, filamentous appendages arising from near the posterior extremity of the body. Flagella four, anterior, active.

*H. inflata* (Fig. 13) was found in water containing *Ceratophyllum* just beginning to decay.

### ORDER EUGLENIDA

GENUS CRYPTOGLENA Ehrenberg.

Body oval, not changeable in form, nor varying greatly among the members of the species; possessing one greenish yellow, or greenish brown chromatophore, or two. Flagellum stout at its base, there may be present a red stigma. Swims rapidly, with an undulatory course.

Two species, *C. conica* (Fig. 14) and *C. pigra* (Fig. 15), seem to be quite common in pond waters among such smaller aquatics as *Lemna, Elodea,* etc. They are often found in water in which there is decaying vegetation, also, associated with *Euglena* and *Phacus.*

GENUS NOTOSOLENUS Stokes.

Body hollowed, resembling the bowl of a blunt, thick, wooden spoon; hyaline, colorless, and filled with a large number of large, globular, glassy granules or vacuoles. We have observed but very few individuals which did not contain these. Flagellum long, stiff, and stout, and rigid except that its distal fourth only is motile. This pecu-

liar characteristic affords a good identification character. During swimming it often turns over and over in the water, at which times the concavo-convex shape of the body becomes appreciable.

Either individuals of the species *N. orbicularis* (Fig. 16) vary remarkably in size, or there is more than one species, differing, apparently, only in this particular. The form of the body is, however, constant.

These forms are found, unlike most of the flagellata, most abundantly in fresh waters, particularly in the clear cold waters of springs, that support little plant growth, and they occur usually near the bottom, among the sands and pebbles.

Their progression through the water is often slow and deliberate, and at such times it seems as though the tip of the flagellum were functioning as an exploratory antenna.

Formulary of Reagents Used for Retarding, Killing ang Staining

1. Retarding Solutions:
   (1) Gelatine Solution.
   Water ................................. 5 oz.
   Gelatine ................................. ¼ oz.
   Heat, to dissolve the gelatine; then allow to cool to the desired viscosity.
   (2) A 1 per cent. aq. sol. of chloretone narcotizes the animals, and gave good results with certain forms, though its manipulation was a trifle difficult.
2. Killing Reagents:
   (1) 25 per cent. aq. sol. tannic acid.
   (2) 5 per cent. aq. sol. acetic acid.
   (3) 10 per cent. aq. sol. mercuric chloride.
   (4) 1 per cent. aq. sol. formalin.
   (5) Invert slide with its suspended drop over the neck of a bottle containing a 2 per cent. sol. of osmic acid. The fumes kill the animals at once.
   (6) 1 per cent. aq. sol. copper sulphate. This gave the best results of any of the killing reagents.
3. Stains:
   *A.* For *intra vitam* staining. (Make up a quantity of the stain as directed, and then dilute with water to obtain desired depth of color.)

(1) Sat. aq. sol. methylene blue.
(2) Sat. aq. sol. methyl green.
(3) Sat. aq. sol. gentian violet.
(4) Sat. aq. sol. safranin.

*B*. For staining after killing, or for killing with the stain itself. (Make up a quantity of the stain, and dilute with water for desired color.)

(1) Sat. alcoholic sol. methylene blue.
(2) Sat. alcoholic sol. methyl green.
(3) Sat. alcoholic sol. gentian violet.
(4) Sat. alcoholic sol. safranin.
(5) Sat. alcoholic sol. iodine, with 3 per cent. potassium iodide. This gives excellent results. It is a very powerful stain, and must be used in weak solutions.

# NEOTENY[1] AND THE SEXUAL PROBLEM

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It has long been known that the larvæ of certain Urodeles sometimes fail to undergo metamorphosis, yet become sexually mature in the larval stage. Perhaps the best known of such cases of neoteny, as this phenomenon is called, is that of the Mexican axolotl, long regarded as a separate species, now known to be an overgrown, sexually mature larva of *Amblystoma tigrinum*. Several other similar cases have been described, however. All neotenous amphibians hitherto reported, with the exception of Allen's (1) thyroidless tadpoles, have been confined to the tailed amphibians, and so far as the writer is aware, the normal occurrence of precocious ripening of the sex cells in larval Anura has never been described. Oddly enough, it is the normal thing, and its occurrence throws considerable light upon the obscure problem of sex differentiation and development in the Anura, which has long puzzled investigators of this subject. It will be recalled that Pflüger (5) reported years ago, that there occurs normally in newly metamorphosed frogs three kinds of individuals, males, females and hermaphrodites, the two latter forms much more numerous in early stages than the males. In the course of further development the hermaphrodites become either definitely male or female, as the sex ratio for adult frogs is approximately 50–50. The investigations of R. Hertwig, Kuschakewitsch and Witschi (2) not only confirmed Pflüger's work, but ex-

[1] The choice of the word neoteny is perhaps not a fortunate one but because it has come to be associated with the attainment of sexual maturity in the larval stage, it will be employed here in that sense. Literally, neoteny means the prolongation or extension of the period of youth, and it has no necessary relation to sexual conditions.

tended it by showing that anurans apparently first develop solely as females and sexual intermediates, the males only later differentiating from the females and hermaphroditic forms. Moreover, these investigators described in great detail modification of the sex ratios by environmental changes such as extremes of temperature and late fertilization. All of these alleged facts have given rise to the belief that anurans in their sexual development differ greatly from other vertebrates.

For several years the writer has been engaged in studying the germ cells of anurans, more especially *Rana catesbiana,* with the object of testing the theories of sex differentiation and development of the Pflüger-Hertwig-Kuschakewitsch school of German investigators. Although at first inclined to admit their contentions, a more careful survey of my material revealed several facts irreconcilable with their views, but which could not be satisfactorily interpreted. Fortunately an opportunity presented itself of working with Professor E. G. Conklin who suggested, after examining my material, that I was probably dealing with a case of precocious ripening of the germ cells in anuran larvæ, *i.e.,* condition stimulating neoteny, using this word in the sense applied above. The suggestion proved correct, and it is a pleasure to acknowledge my indebtedness to Professor Conklin for giving the clue to correct interpretation of the problem and for many other helpful suggestions as well. The present paper is a brief summary of a more extensive and detailed investigation scheduled for later publication.

In *Rana catesbiana* the larval period is very long, some few individuals requiring four seasons to complete metamorphosis, though the usual period is about two years. The sex of larvæ 55–65 mm. in length is not difficult to determine by examination of the gross structure of the gonads, but if such superficial examination is supplemented by a hasty survey of the microscopic appearance of the germ cells, then oddly enough hopeless confusion of the sexes results, and what were apparently males from macroscopic evidence turn out to be apparent fe-

males. The sex ratios will vary greatly according to the stress laid by the observer on the gross appearance of the gonads (and it must be admitted that in early stages the gonads of the two sexes are remarkably alike) or upon the cytological evidence as it has heretofore been interpreted. In larvæ of 80–100 mm. length the sex ratio is approximately 50–50 when based on the evidence presented by the gross appearance of the gonads; on the other hand, the cytological criterion, as it has been interpreted, practically does away with males, while most of the animals are apparently female. It is probably due to this erroneous interpretation of cytological conditions that such confusion reigns in the literature regarding sex in anurans.

The germ cells of larvæ 45 mm. and over, both *male* and female, are found in early maturation stages. Such animals are about 8 months of age. Practically all of the female cells are in the leptotene or pachytene stage. In the females the leptotene and pachytene stages do not persist for any length of time, but give place to the period of growth, in which the cells with pachytene nuclei increase greatly in volume, are invested by a follicle of peritoneal cells, and become typical oocytes. Those cells bordering the lumen of the gonad, are first to enter the growth period, and by reason of their great increase in size, fill up the cavity. Around the periphery of the gland a ring of cells with leptotene and pachytene nuclei persists, giving rise later to oocytes of a younger generation. Scattered through the gland are a few oogonia with polymorphic nuclei.

The female gland increases greatly in size due to the growth of the oocytes, becomes much infolded and convoluted by inequalities of growth, thus taking on the characteristics of the typical ovary of the adult. These typical ovaries are to be found in larvæ over 80 mm. long. The gonads probably persist in this condition for several years after metamorphosis, the oocytes growing very slowly. According to the observations of Hertwig and others, the females of *Rana temporaria* and *Rana escu-*

*lenta* do not become fully mature and ready for copulation until the fifth season after metamorphosis. The writer has captured two females of *Rana catesbiana* two seasons after metamorphosis which were yet sexually immature, hence it seems that the females of this species also require a long period of time in which to develop sexual maturity. The developmental history of the male gonads and germ cells is quite different, and when rightly interpreted fails to show female animals transforming into males and vice versa, or abnormal sex ratios.

It was stated that germ cells of the male larvæ begin their maturation cycle simultaneously with those of the females. This is a very unusual condition and probably unique among the vertebrates though common enough in the invertebrates. It will be recalled that in the vertebrates—for example the mammalia, a very long period of time, sometimes years, separates the maturation cycle of the sexes. In many instances in the female, the initial maturation changes preceding the growth period of the oocyte, occur before birth, whereas the same nuclear changes in the male cells do not make their appearance until shortly before the attainment of sexual maturity. It has become the custom for investigators of sexual conditions in the Anura to use this fact of the early occurrence of maturation stages preceding the growth period of the oocyte in the female as a cytological criterion for differentiating the sexes in the larval stages. Unfortunately this principle, though true enough for other vertebrates is not applicable to Anurans and the result has been hopeless confusion of the sexes because in this group maturation occurs in larval males.

From the period of formation of pachytene nuclei, the history of the sexes is quite different in *Rana catesbiana* and unmistakable if a complete series of larval stages is obtained. In justice to other investigators whose results the writer criticizes as based upon misinterpretation of sexual conditions, it is fair to point out that of all existing species of Anura, *Rana catesbiana* is apparently the only one in which precocious ripening of the male germ

cells goes as far as the formation of the maturation division in first year larvæ, and ripe spermatozoa in second year animals. This is of course due to the extraordinarily long larval period. In *Rana temporaria* and *Rana esculenta* the larval maturation changes apparently go only up to and including the pachytene stages before degeneration sets in. In Bufo, the precocious ripening of the sex cells is confined entirely to the cells of Bidder's organ and continues up to the pachytene stage before growth begins.

The male larvæ of *Rana catesbiana* undergo two distinct seasonal maturation cycles as larvæ. The first occurs in young animals 45–60 mm. total length, despite the fact that the germ gland is in an extremely undifferentiated condition. The germ cells develop normally through the leptotene, pachytene, diplotene and tetrad formation stages, but invariably degenerate and go to pieces during the late metaphase or early anaphase of the first maturation division. The centrosomes fragment and the spindle apparatus is aberrant. There are no second maturation divisions, though occasional giant spermatid-like structures may form by the growth of axial filaments from the centrosomes of first spermatocytes. The cells of the first larval maturation cycle degenerate. Through active mitotic division the few primary spermatogonia scattered throughout the gland give origin to those cells which later undergo the second larval sexual cycle. This second cycle occurs near the end of larval life, *i.e.*, usually about two years after hatching. Oddly enough the second maturation cycle is normal, and gives rise to functional spermatozoa in the larvæ, though the efferent ducts of the testes are not yet fully formed. The germ cells and tetrads of the first sexual cycle are aberrant in size and character, those of the second cycle are normal in every way.

The diploid chromosome number of the larvæ is twenty-eight, the haploid number is fourteen. There is no evidence of an accessory chromosome.

Probably the larval sexual cycle just mentioned is an interesting example of a "carrying over" in ontogeny of an earlier phylogenetic condition when the Salientia were sexually mature and normally reproduced as larvæ. It is interesting to note in this connection that male anuran larvæ whose period of metamorphosis is indefinitely postponed, as for example by thyroid extirpation, readily mature sexually, in so far as the production of ripe spermatozoa is concerned.

The male germ cells, unlike those of the female, do not undergo growth, except in relatively rare instances to be described later, and consequently do not fill up the lumen of the gonad. This lumen later is obliterated by the migration into the gonads of cells from the mesentery and possibly from the cortical substance of the adrenal gland. From this ingrowth the testicular interstitium and rete apparatus develops. The efferent tubules at the time of metamorphosis form a connection with the mesonephros. The true sex cords of the testis arise as proliferations of the germinal epithelium, and not as so often claimed for amphibia, as ingrowths from the mesonephros.

This phenomenon of precocious ripening of the male germ cells of *Rana catesbiana* larvæ undoubtedly occurs in other Anura, though is not carried so far as in the bullfrog. The figures of Kuschakewitsch and Witschi show clearly that this condition exists in *Rana esculenta* and *Rana temporaria.* Indeed, it seems more than likely that these writers have mistaken male frog larvæ whose germ cells were in early pseudo-reduction stages, for hermaphrodites and females. The so-called sexually indifferent or sexually intermediate forms of the Pflüger-Hertwig school are very probably male animals whose germ cells show precocious ripening as far as the pachytene stage. This is plainly evident from their photographs, drawings and descriptions. This probable misinterpretation of the cytological data accounts for the transformation of such so-called hermaphrodites into male animals, so minutely described by these investigators. Using the chief cri-

terion of sex differentiation employed by Witschi, *i. e.*, that all germ cells in the larval gonads showing pseudo-reduction (leptotene and pachytene stages) are to be regarded as female, the writer obtains in his set of 2,000 animals, 96 per cent. females and only 4 per cent. males. In larvæ of 85 mm. length the percentage of males is zero if this criterion of sexual differentiation is employed. The writer has found so far no evidence that the sex ratios of Anure are any different from those of other vertebrates, and is inclined to regard the confusion concerning sex differentiation and development in anurans as a result of interpretating male animals showing precocious maturation changes of the germ cells as females and hermaphrodites. Sex in the frog does not appear to be nearly so labile and easily influenced as some investigators claim. Professor Hertwig's "late fertilization" experiments are more satisfactorily interpreted on the chromosomal hypothesis of sex determination, than on any other.

One reason so many workers dealing with anurans have regarded these animals as possessing hermaphroditic tendencies is the occurrence of "oocytes," so-called, in the testes of larval and adult frogs and the presence of the peculiar ovary-like structure, the organ of Bidder, in the Bufonidæ. The origin of these apparent oocytes in *Rana catesbiana* has not yet been worked out as completely as the writer could wish; however, enough data has been collected to warrant a tentative explanation of their occurrence in this form and the same data is suggestive as regards the nature of Bidder's organ in the male toad, at least suggestive enough to warrant a reinvestigation of this structure, now generally regarded as a rudimentary ovary.

In male *Rana catesbiana* larvæ, these large oocyte-like cells are of frequent occurrence, and assume this character while in the pachytene stage. Previous to the growth period they are indistinguishable from the other pachytene male cells of the gonad. During the growth stage, which is later followed by their degeneration and

disappearance, they are similar in every way to the cells of Bidder's organ. Their follicles are derived from surrounding peritoneal or stroma cells. These follicles are commonly observed surrounding isolated spermatogonia. In certain male gonads a few cells grow to the size of oocytes, and possess yolk nuclei. The presence of yolk, however, is no sex criterion for the male germ cells of many animals form yolk as for instance *Ascaris,* and the apyrene spermatozoa of certain Prosobranchs, and the degenerating cells of the frog.

The larval spermatocytes of the first maturation cycle are in many instances of enormous proportions, scarcely smaller than many organs of Bidder cells near the end of their cycle. It is not impossible that there may be a genetic relation between these two types of testicular elements. This question is reserved for further discussion in a later paper.

The writer is of the opinion that these "oocytes" are of the same nature as the cells of Bidder's organ in *Bufo.* It might be suggested as a possibility worthy of consideration, that in male animals such cells may be of true male character, but owing to the precocious sexual cycle, itself a vestige of a primitive phylogenetic condition when the Anura were sexually mature in the larval form, a few of the germ cells are unable to complete their cycle, and simply grow to an abnormal size, thus assuming the unspecialized character associated with oocytes. These cells degenerate during the second larval maturation cycle when normal sex products are produced. It is rather significant that the whole first larval sexual cycle is abortive in almost every feature. For instance the spermatocytes are abnormally large, the tetrads equally so, the first maturation division never proceeds past the anaphase, the centrosomes fragment, form polyasters, and sometimes axial filaments. Entire cysts of perfectly formed spermatocytes go to pieces in the very act of division, and most of the germinal elements show marked evidences of a deep seated protoplasmic disorganization.

In view of these facts it is possible that the cells of

Bidder's organ in *Bufo,* and the oocytes-like cells of anurans may not be true oocytes, despite their appearance, but may be merely senescent cells, occurring in the course of an abortive and degenerate larval sexual cycle. Bidder's organ on this assumption is the vestigial remains of a primitive sex gland functional when the Bufonidæ reproduced in the larval stage. The functional gonads of present day toads represent recently acquired structures superimposed upon the phylogenetically older and degenerate glands.

In *Rana catesbiana,* a more primitive anuran type than *Bufo,* the entire larval male gonad might with some plausibility be compared to an organ of Bidder in which only a few cells assume the oocyte character whereas the remainder develop a little further, *i.e.,* to the first maturation anaphase, when they too go to pieces. The writer suggests this view of Bidder's organ tentatively, and pending further investigation does not regard himself as irrevocably committed to it. The facts are suggestive, and that is all that can be said at present.

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# SHORTER ARTICLES AND DISCUSSION

## THE TABULATION OF FACTORIAL VALUES[1]

In *Science* for January 23[2] Dr. Ellis L. Michael discusses the validity of the ordinary system of tabulation in the determination of the probable number of bacteria in an emulsion. He argues in favor of the use of the logarithms of the measurements instead of the direct measurements because the former give a symmetrical distribution, while the latter give one that is distinctly asymmetrical. As Dr. Michael has invited discussion it may be of interest to mention briefly a similar method used during the last two years in a study of the germinal and environmental factors affecting eye facet number in the bar races of *Drosophila*. A report of the method was made at the St. Louis meeting of the American Society of Zoologists and the results of its application to the particular problems in hand are being published in a series of papers.[3]

In working up the data it became evident that the demands of the biological analysis were not adequately met by the system of arrangement in classes with equal facet numbers. The wide range in individual stocks and the still wider differences between different races made it desirable to express relations directly in terms of factorial units affecting facet number rather than in facet numbers. In dealing with a stock averaging 30 facets as compared with one averaging 300 facets it became evident that a one facet change at the mean in a 30 facet stock represents the same factorial value as a ten facet change at the mean in a 300 facet stock and that *a corresponding principle applies within the range of a single stock.* Accordingly the classes were arranged

[1] Contribution from the Zoological Laboratory of the University of Illinois, No. 152.

[2] "Concerning Application of the Probable Error in Cases of Extremely Asymmetrical Frequency Curves," *Science*, N. S., 51: 89–91.

[3] "A Change in the Bar Gene of *Drosophila* Involving Further Decrease in Facet Number and Increase in Dominance," *J. Gen. Physiol.*, 1919, 2: 69–71. *J. Exp. Zool.*, 1920, 30: 293–324.

so that the facet range of each class is a fixed per cent. of the mean facet value of *its class.* In other words the class facet ranges vary in such a way as to give the same logarithmic range to each class.

As an illustration eye facet counts in 488 females of the unselected white bar stock may be taken. The following table gives the frequency distribution obtained when the classes have the same facet ranges:

| Facet Counts | Frequency in Per Cents. |
|---|---|
| 16– 21 | 0.2 |
| 22– 27 | 0.2 |
| 28– 33 | 2.9 |
| 34– 39 | 10.9 |
| 40– 45 | 14.3 |
| 46– 51 | 12.3 |
| 52– 57 | 12.9 |
| 58– 63 | 11.7 |
| 64– 69 | 9.2 |
| 70– 75 | 8.0 |
| 76– 81 | 3.9 |
| 82– 87 | 3.9 |
| 88– 93 | 3.3 |
| 94– 99 | 2.9 |
| 100–105 | 1.4 |
| 106–111 | 1.6 |
| 112–117 | 0.2 |
| 118–123 | 0.0 |
| 124–129 | 0.0 |
| 130–135 | 0.2 |

The same arrangement is shown in graphic form in the following figure:

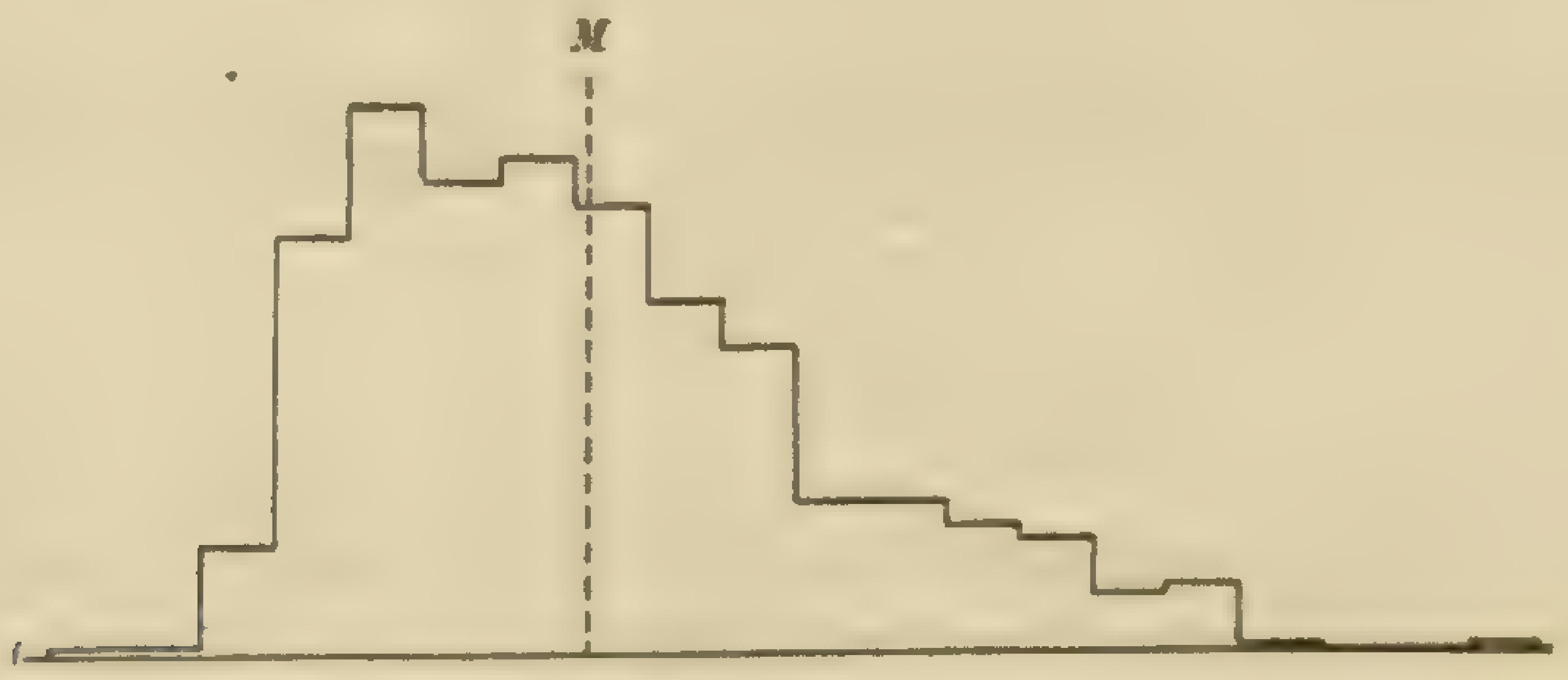


FIG. 1.

There is a marked positive skewness. The next table uses the same original data but with the facet range in any class equal to ten per cent of the mean of *that* class:

| Facet Counts | Frequencies in Per Cents. | Factorial Units from Mean |
|---|---|---|
| 20– 21 | 0.2 | —9.93 |
| 22– 23 | 0.0 | —8.93 |
| 24– 26 | 0.2 | —7.93 |
| 27– 29 | 0.6 | —6.93 |
| 30– 32 | 1.8 | —5.93 |
| 33– 35 | 3.1 | —4.93 |
| 36– 39 | 8.2 | —3.93 |
| 40– 43 | 9.6 | —2.93 |
| 44– 48 | 11.1 | —1.93 |
| 49– 53 | 11.9 | —0.93 |
| 54– 59 | 11.1 | +0.07 |
| 60– 65 | 11.3 | +1.07 |
| 66– 72 | 10.4 | +2.07 |
| 73– 80 | 5.9 | +3.07 |
| 81– 88 | 5.1 | +4.07 |
| 89– 97 | 5.5 | +5.07 |
| 98–107 | 2.3 | +6.07 |
| 108–118 | 1.4 | +7.07 |
| 119–131 | 0.0 | +8.07 |
| 132–145 | 0.2 | +9.07 |

The following figure is based on the same arrangement:

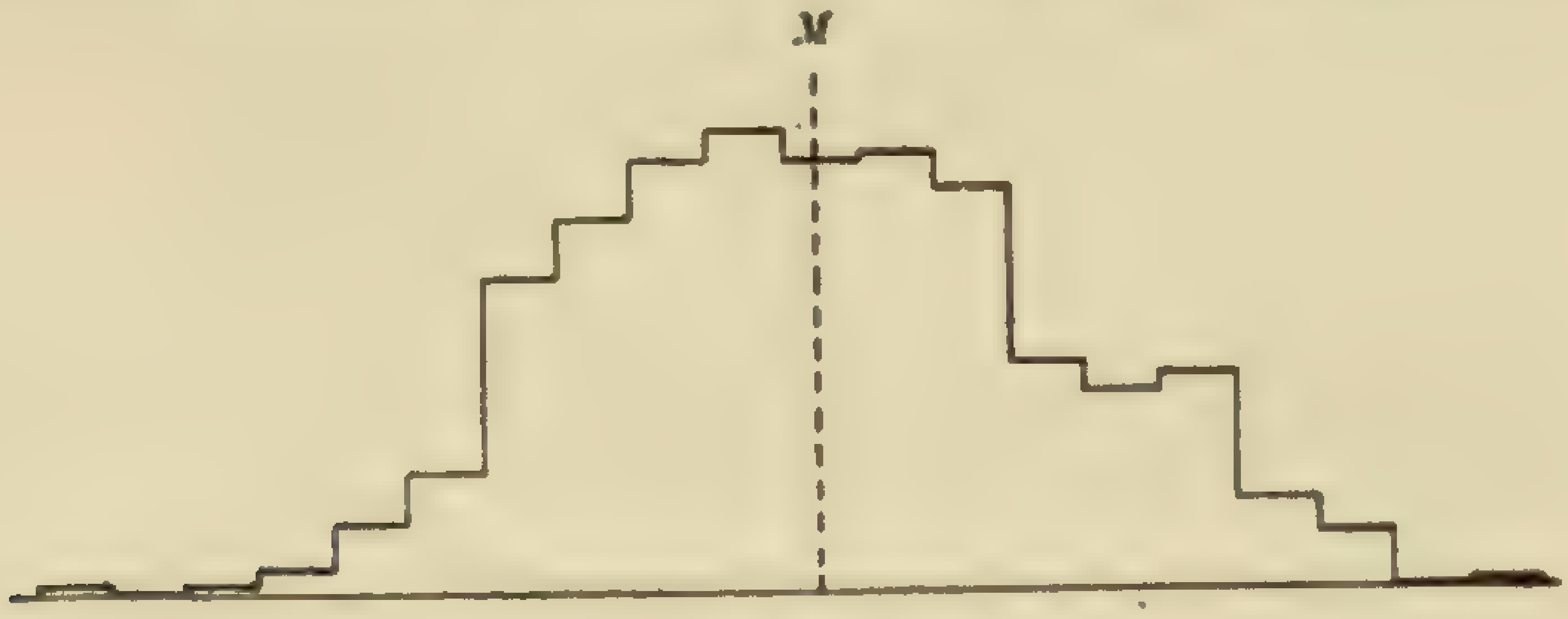


FIG. 2.

This is much closer to a normal distribution of frequencies than in the ordinary method. It is correspondingly more reliable in the determination of the various constants.

If the biological assumption upon which this tabulation is based is correct the classes are of equal value as far as the *factors* affecting facet numbers are concerned though the facet ranges are different. In following out this view the intervals on the variation scale have been expressed in terms of class units, each unit being equivalent to a factor which produces a change of ten per cent. in facet number. Some arbitrary point, for instance the mean of the unselected stock, may be taken as the point of reference or zero and every facet value has a corresponding factorial value on the scale. The variation constants may be ob-

tained in the ordinary way but in terms of factorial units and not facet units. The standard deviations are used directly as coefficients of variation.

The biological validity of the factorial method as given is of course dependent upon the correctness of the view that eye facet numbers have such a relation to environmental and germinal factors as is indicated. The normality of the factorial distribution has already been mentioned. General embryological considerations favor proportionate action of factors rather than action by accretion. But I wish to mention particularly the definite experimental proof that at least one factor, temperature, is in agreement with the hypothesis. Seyster[4] has shown that in bar eye facet number decreases with increase in the temperature at which the larvæ of *Drosophila* are reared. This decrease follows van't Hoff's law if an inhibitor of facet number is assumed as the effective agent upon which the temperature acts. Krafka[5] has demonstrated that this general law applies to ultra-bar as well as to bar eye and that for the different bar stocks the effect of a degree of change in temperature is roughly proportional to the mean value of the stock and the same is approximately true for the effects of a degree of change in temperature throughout the range of a single stock. The following table gives the facet values for ultra-bar, low selected bar and unselected bar at 15° and 25°:

| 15° Facet Values | 25° Facet Values | Differences | Ratios of Differences |
|---|---|---|---|
| 51.5 | 25.2 | 26.3 | 1.0 |
| 189.0 | 74.2 | 114.8 | 4.4 |
| 269.8 | 120.5 | 149.3 | 5.7 |

Representing the effect of a ten-degree difference for ultra-bar as unity, low selected bar has 4.4 times and unselected bar 5.7 times this difference. It is obvious that difference in facet number is not a good measure of the value of the temperature factor.

On the other hand, if facet values are reduced to factorial values according to the method given above the results are as follows:

[4] Seyster, E. W., ''Eye Facet Number as Influenced by Temperature in the Bar-eyed Mutant of *Drosophila melanogaster* (*ampelophila*),'' *Biol. Bull.*, 1919, 37: 168–182.

[5] Krafka, Joseph, Jr., ''The Effect of Temperature upon Facet Number in the Bar-eyed Mutant of *Drosophila*,'' *J. Gen. Physiol.*, 1920. (In press.)

| 15° Factorial Values | 25° Factorial Values | Differences | Ratios of Differences |
|---|---|---|---|
| − 0.83 | −7.86 | 7.03 | 1.0 |
| +12.17 | +2.79 | 9.38 | 1.3 |
| +15.72 | +7.73 | 7.99 | 1.1 |

This is a much closer approach to unity for the ratios than in the case of facet values and the units employed may be taken as fairly close measures of the temperature factor.

A change of one facet is therefore not of equal factorial value at different points on the variation scale as far as temperature is concerned. A plotting of the data using facets as the units does not give a uniform factorial scale. Suppose temperature to be the only factor causing variation in the facet number of a particular stock but knowledge of the actual temperatures involved in the production of a particular population to be lacking and it is desired to derive the value of the temperatures from the facet values. Obviously the closer approximation is obtained by the tabulation in which each class has a facet range equal to a definite per cent. of its facet mean. Krafka's data show that even in this case the determination is not exact but certainly the error is of a much lower order than that involved in using facets as the units.

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## AN EXPERIMENT ON REGULATION IN PLANTS[1]

IT is a fundamental fact that of the enormous number of buds on a tree only a few of these normally develop into branches. Every bud, however, has the capability of growth and will grow into a branch if the more apical bud or buds are removed. Even normally, in uninjured trees, some of the lateral buds grow into

[1] After this paper was written, my attention was called to an article by Child and Bellamy (*Science,* N. S., L, 362, 1919), in which somewhat similar experiments were reported and the same conclusion arrived at. Physiological isolation of two regions of a whole plant was produced by low temperature instead of by actual killing of tissue as in my experiments. In view of the importance of growth phenomena I believe it worth while to again call attention to the conclusions to be drawn from these facts, especially as the experiments of Child and Bellamy refer only to the influence of a growing stem on the growth of other stems and not to the influence of growing roots on the development of roots in other regions of a plant.

branches and the characteristic form and type of growth of a plant are thus determined. It is a species characteristic.

An analysis of the factors which retard the growth of lateral buds can best be made on plants with only a few buds, and an excellent discussion of the problem has been given by McCallum.[2] McCallum worked with the scarlet runner bean, *Phaseolus multiflorus.* The cotyledons of this bean remain at the surface of the ground and the buds in their axils never develop unless the growing stem is injured or removed. Then they invariably develop and form shoots. No amount of wounding short of removal of the growing tip will cause these buds to grow. They never grow if the terminal bud is present, no matter how much food or water is available with optimum light and temperature conditions, and they always start to grow if the terminal bud is removed and at the same time the plant is starved by cutting off cotyledons and root systems or is practically dehydrated by placing it in a very dry atmosphere.

To describe the phenomenon we say that the tip inhibits the growth of buds below it. Only a *growing* tip has this inhibitive action, for McCallum showed that if the tip is kept in a hydrogen atmosphere, which prevents its growth, the cotyledonary buds begin to grow. Later if the tip is removed from the hydrogen to air, it also will again grow.

The inhibitory influence of a growing stem tip on latent buds is exerted only downward. A growing root exerts an inhibitory influence on the development of roots above it and this influence passes upward along the stem. If the roots of a bean plant are removed, new roots will develop along the stem wherever there is most moisture. The new roots do not come from root buds as the new shoots come from shoot buds, but they arise from unformed regions of the stem. The inhibitory influence on root formation which passes upward moves along the vascular bundles and is restricted to that section of the stem immediately above in line with the point of injury to the root. This, if the stem of a bean plant is kept moist and a small notch is made (so as to cut the vascular bundle) in the stem below this moist region, the inhibitory action of the main roots will be cut off by the notch and secondary roots can now form in this moist region only on the side above the notch (see Fig. 10, p. 116 of McCallum's paper).

The inhibitory action of a growing tip in the bean on buds

[2] McCallum, *Bot. Gaz.*, XL, 97 and 241, 1905.

below is not localized in the stem perhaps we may say is not transmitted in a direct line in the stem. A notch cut half-way across the stem will not cause the cotyledonary but directly below it to grow. In *Bryophyllum,* however, Loeb's[3] experiments have indicated that the inhibitory influence of a leaf on the growth of axillary buds passes downward in the same sector of stem as the leaf itself. Moreover the inhibitory influence appears to be of the nature of material flowing, because the pathway of the inhibitory influence is affected by gravity. This is illustrated in Figs. 11, 12 and 13, pp. 349 and 351 of Loeb's paper. This influence of gravity is a fundamental fact whose importance for the explanation of regulation phenomena must not be overlooked.

It is obvious to one who seriously contemplates the facts of regulation that the influence of one part over another in the organism must be either similar to nerve influence and depend on living protoplasmic continuity between the parts, or due to the actual transport of material from one region to another. Unless we are to assume the existence of a guiding all powerful form-determining spirit or force, which is as difficult to prove as to disprove, there is no other than these two explanations. These we know to be two means of "action at a distance" in animals and we might expect them to be operating in a plant also.

Let us consider the second of these possibilities—transport of material. Two views are prevalent regarding the nature of this transport. (1) A growing stem may be supposed to form material which inhibits shoot formation and a growing root to form material which inhibits root formation.[4] These special inhibition substances pass downward and upward in the stem respectively. Polarity is a direct consequence of the formation of these substances and their direction of flow. Loeb has pointed out several instances of growing roots inhibiting the formation

[3] Loeb, J., *Journ. Gen. Physiol.*, I, p. 337 and 687, 1919.

[4] It has been suggested that a growing stem forms material which inhibits shoot formation but favors root formation. The root formative substances collect at the basal end of a cut stem and induce the formation of roots there. That this assumption will not hold is indicated by McCallum's experiments on root formation already mentioned. (Fig. 10, p. 116.) The stem of a whole bean plant is surrounded near its upper end by water held in a glass vessel. This gives favorable conditions for the development of roots but none grow so long as the roots of the plants are intact. If they are cut off new roots form, not at the base of the stem where the cut is and where root forming substances should collect, but in the water high up on the stem.

of roots in other regions of a plant of *Bryophyllum* and of even stopping the further growth of roots which had started to grow.

(2) In a whole plant, because of a certain morphological structure, the nutrient channels are such as to carry food material to growing regions. A plant grows at both ends and so long as these are intact and growing food flows toward them. If removed, food flows to other points and starts the growth of dormant short buds or root primordia. Once a stem or root has started growing Loeb's[5] experiments show very clearly that the mass of growth formed is proportional to the mass of materials available.

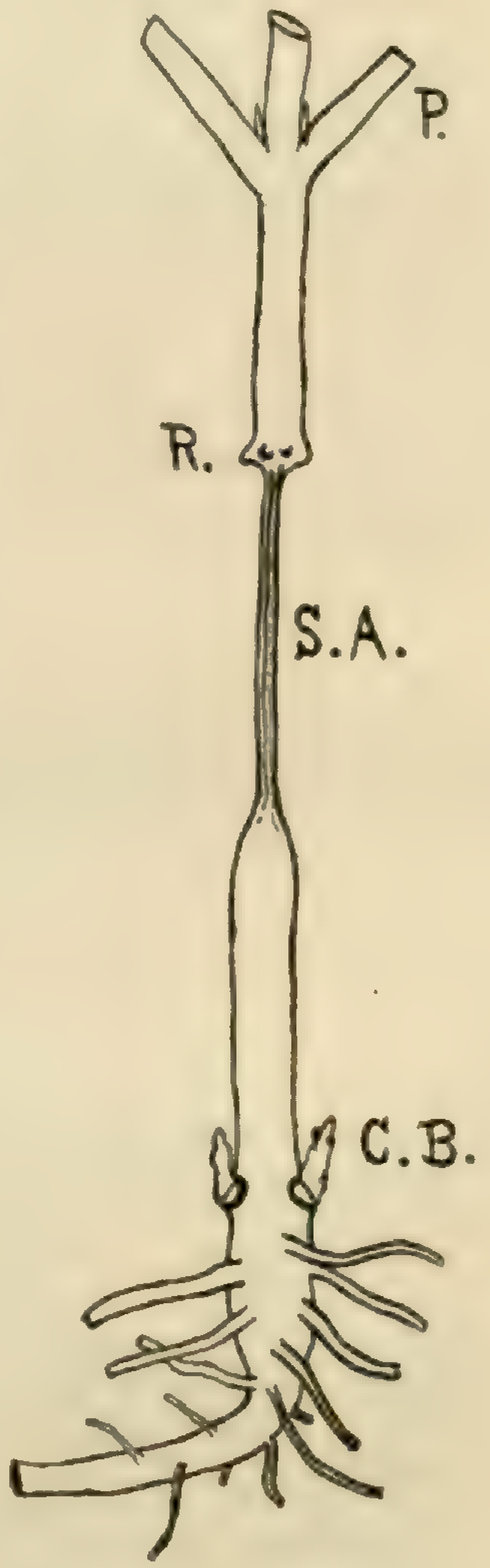


FIG. 1. Drawing showing condition of a bean plant eight days after the stem has been steamed. The top and tap root have been cut off to reduce its size for preservation. They were alive and growing during the experiment. Note that the cotyledonary buds (C. B.) grow below the steamed area (S. A.), and roots (R) appear above the steamed area. P, petioles of first pair of leaves.

I believe that light is thrown on this problem by some experiments which I performed in 1910, while a student at Columbia University, and repeated at Princeton in 1912, but which have never been published. They are designed to divide a plant into two parts physiologically but not morphologically. A jet of steam was directed against the stem of a young bean plant between cotyledons and first pair of leaves in order to kill the tissue throughout the stem in this region. In some plants the leaves and growing tip above this region wilt and die but in many cases not only does no wilting occur but the tip continues to grow and *as rapidly or more rapidly* than control plants under the same conditions which are unsteamed. Nevertheless the *cotyledonary buds below the steamed region begin to grow and roots start to appear just above the steamed region.* If the air were sufficiently moist or the region surrounded by water there is no reason why these incipient roots should not grow out into a typ-

[5] Loeb, J., *Journ. Gen. Physiol.*, I, p. 81, 1918.

ical root system. I have preserved the stems of plants steamed in this way and Fig. 1 is a drawing of one of these. It will be noted that the steamed region, which was exposed for three minutes in this plant, has shrivelled to a hard woody connection about 24 mm. long. Table I gives the data regarding the growth of control and steamed plants whose tops were not killed by the steaming. The average growth for the three controls is 40.5 mm., and for the steamed plants, 59 mm. It is evident from the table also that the terminal bud has grown in the 24 hrs. immediately after steaming so that we cannot say that the steaming caused even a temporary cessation of growth of the tip.

TABLE I

RATE OF GROWTH OF STEAMED AND CONTROL BEAN PLANTS

| | Plant | Time Steamed in Seconds | Length of Shrivelled Stem in Mm. | Mar. 7, Length of 1st Internode Above Leaves | Mar. 8, Length of 1st Internode Above Leaves | Mar. 11, Length of Internode | | Mar. 15, Length of Internode | | Total Growth in 8 Days, in Mm. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 1st | 2d | 1st | 2d | |
| Controls, unsteamed. Cotyledonary buds do not grow. | 1 | | | 1.5 | 1.5 | 7.5 | | 26.0 | | 24.5 |
| | 2 | | | 4.0 | 6.0 | 16.5 | 3.5 | 25.0 | 14.0 | 35.0 |
| | 3 | | | 5.0 | 10.0 | 34.0 | 2.0 | 50.0 | 17.0 | 62.0 |
| March 7, 1912. Steamed. Cotyledonary buds all grow. | 1 | 5 | 8.0 | 2.0 | 5.0 | 20.0 | | 38.0 | | 36.0 |
| | 2 | 7 | 5.5 | 5.0 | 7.5 | 9.0 | | 21.0 | | 15.5 |
| | 3 | 10 | 9.5 | 7.0 | 11.0 | 39.0 | | 69.0 | 11.0 | 73.0 |
| | 4 | 15 | 15.0 | 14.0 | 24.0 | 60.0 | 14.0 | 66.0 | 47.0 | 99.0 |
| | 5 | 20 | 14.0 | 5.0 | 8.0 | 37.0 | | 71.0 | 20.0 | 86.0 |
| | 6 | 180 | 24.0 | 13.0 | 18.0 | 47.0 | | 48.0 | 10.0 | 45.0 |

It is certainly true that sap must pass up the stem of these steamed plants, otherwise the tops would remain turgid and growth occur. Root inhibiting substances, if formed, must have passed upward along with the sap. Nevertheless we find roots developing above the steamed region despite the fact that the plant has a normal living root system below. The evidence is conclusively against the existence of definite root inhibitive substances. If sap can pass upward in a steamed area we might expect that it could pass downward also. If inhibitive substances are formed by a growing stem these materials should reach the cotyledonary buds below. Nevertheless these buds develop. Since we can not necessarily argue that because material can pass up a stem it must also pass down, the evidence points against the existence of shoot inhibitive substances, but is not unequivocal.

In a plant which has been steamed the nutrient channels are the same as in a normal plant. The apical bud is growing and attracting material to it so that we cannot say this food material is now available for the cotyledonary buds as we might had the growing tip been actually cut off or prevented from actively growing by a hydrogen atmosphere. The evidence is conclusively against the view that growing points prevent the growth of dormant buds by attracting and utilizing the nutrient material.

It would seem that the inhibitive influence must be dependent on living functioning protoplasmic connections. How are we to conceive of an influence of this sort without invoking a vitalistic explanation? I believe the explanation lies in the direction developed at length with the aid of metal models by Lillie.[6] Growing points are of a different electrical potential as compared with other points and the currents so generated passing through dormant buds in the proper direction, prevent their growth. The potentials are phase boundary or membrane potentials, possibly dependent on selective ionic permeability or solubility of two phases (cell and medium) to ions, and consequently dependent or normal permeability conditions throughout the plant. Interruption of living protoplasmic connections, then, means merely the interruption in continuity of semipermeable membrances in longitudinal axes of the plant (vascular bundles?). While we may be sure that the steamed portion of a plant will conduct an electrical current, since its normal semipermeable membranes have been destroyed there is no means of obtaining a return circuit. The plant is divided into two electrical systems instead of one and behaves practically as two distinct plants. As Lillie[7] has suggested the effect of gravity on the inhibitive influence of growing stems, pointed out by Loeb, may be explained by movement of sap downward and passage of a greater current through this region because of increased electrical conductivity there. Biological polarity thus becomes electrical polarity and a given process at one region or pole is automatically accompanied by the reverse process at the opposite region or pole.

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[6] Lillie, R. S., *Biol. Bull.*, XXXIII, 135, 1917.

[7] Private communication.

## INHERITED PREDISPOSITION FOR A BACTERIAL DISEASE

As soon as it can be demonstrated that in a process under investigation a given factor has a very marked influence, this factor is more often than not looked upon as the sole cause of what happens. It is indeed very difficult not to overemphasize the importance of a new link in a chain of causes, which has been hitherto overlooked, and which one is fortunate enough to discover. To give a few instances from a field familiar to us, we can cite three factors in the evolution of species which have each by one author been elevated to the rank of "the" cause of species formation. Natural selection was the cause of evolution in the eyes of Weismann, and every other factor was looked upon as subordinate. In the same way Wagner overemphasized the importance of isolation, and de Vries would have us believe that mutation was the main, if not the sole, cause of evolution. The greatness of Charles Darwin lies in the fact, that he was not led away from a consideration of all the possible factors by the temptation to pad out the importance of any one link in the chain of causes.

In a few instances the discovery of a new and very important factor in the causation of a process or set of phenomena sets all the investigators working in the new field just opened up. And often the attention is unduly taken away from other causes. In pathology the discovery of the rôle which microorganisms play in the causation of certain diseases has resulted in the almost absolute neglect of the study of possible other factors in the causation of these same diseases.

In the illness of an individual, infection by a specific microorganism is a very important factor in certain cases. But it is clear that, besides this infection, other factors influencing the qualities of the subject can be of great importance. Very often we find that, besides the presence of the specific organism, predisposing factors play an important rôle, such as the simultaneous presence of another infection (tuberculosis after measles) special conditions (diabetes, possibly beri-beri); causes lowering the vitality (exhaustion, inanition).

Besides factors of the environment, which in themselves are not pathogenic factors, it is evident that factors given in the composition of the individual, inherited factors, can cooperate in the causation of disease.

To make the statement general, we can say that illness is a condition caused by the cooperation of a series of factors, of which some are genetic, heritable, given in the composition of the individual's germ, and others are non-genetic, influencing the individual from the outside. In different combinations of other causes, individual factors can have a very different influence. In certain cases, therefore, different factors can be looked upon as the one which tips the scale, and consequently as "the" pathogenic moment.

The discovery of microorganisms and their rôle in disease has relegated other pathogenic causes to the background, and especially in those diseases where presence of the specific microorganism can always be demonstrated.

In some diseases the presence of a specific microorganism is not demonstrated, and an important non-bacterial factor seems to be the chief determining cause (some cases of carcinoma and of traumatic diabetes). In other cases, presence of a specific microorganism is certain demonstrable, but it seems as if other factors play an important rôle. Tuberculosis is a typical instance. And finally we know diseases, in which it appears as if presence or absence of a specific microorganism constitutes the almost exclusive cause of the difference between affected and healthy individuals (plague).

In the first group, diseases in which microorganisms play no rôle, the factors which cause the abnormal condition can be real environmental factors, but in some instances they are clearly genetic factors, developmental factors transmitted through the germ, genes. We know real hereditary diseases, where an inherited, genotypic peculiarity seems to be the causating factor (hemophily, Huntington's chorea, Daltonism).

In the second group, in those cases, therefore, where predisposition seems to have an influence comparable in its magnitude to infection, this predisposition can have very different causes. In some cases the cause of a predisposition is very clearly non-genetic, environmental (pneumonia after influenza, tuberculosis of the joints after trauma). In other cases, however, inherited constitution is very probably an important factor.

The "inheritance" of tuberculosis has been a point of unending controversy. Very often tuberculosis occurs in families in a way which makes us think of inheritance. According to many authors this occurrence of tuberculosis in families is simply caused by the greatly enhanced chances for a heavy infection. Others

however believe in the possibility of a real inheritance of the disease. It is very evident that the discussion has been very much hampered by a confusion of "inherited" and "congenital." And it has seemed to a great many authorities as if the question as to the existence of an inherited moment in tuberculosis could be answered by an investigation into the possibility of pre-natal infection.

Lastly, there are authors who believe in the inheritance of a certain disposition for tuberculosis.

From the fact that practically all persons above the age of twelve react positively to von Pirquet's test, it can be seen that tuberculosis infection is not as inevitably the cause of tuberculosis, as for instance pneumococcus infection is the cause of septicæmia in the mouse. Every practising physician has seen cases in which a joint became tuberculous after a trauma, in a patient who showed no other evidence of a tuberculous infection. But the fact that such cases are rare makes it probable that constitutional, genetic, differences in resistance exist between individuals. The same holds true for traumatic carcinoma.

It is evident that the study of the inheritance of constitutional predisposition to a disease must be almost impossible, where infection is so general as in the case of tuberculosis. We can only hope to find instances of the inheritance of predisposition or reversely, of immunity to a bacterial disease in cases where we are dealing with one, or with very few genetic factors, genes, whose influence on the resistance happens to be very marked indeed.

Now, in principle, there are reasons to believe in the possibility of an inheritance of immunity or predisposition for bacterial diseases. In the first place we have those instances, in which closely related varieties or species differ in resistance to a specific bacterial infection. A classical instance is that of the Algerian sheep, which are constitutionally immune to anthrax.

Another, similar instance was met by us in our work with rats. We found that there was a striking lack of uniformity in the practical results of the use of a paratyphus culture as distributed by the State Serum-institute of Holland for exterminating rats. In some parts of Holland the broth-culture was highly effective and very well spoken of, whereas it was almost wholly ineffective in other provinces. It appeared to us that this difference might depend upon the species of rats against which the culture was used. It was discovered by some joint work of the Koloniaal

Instituut and ourselves, that the Norway rat, which is the common rat in most parts of Holland, was practically, if not wholly, absent from parts of Friesland. In these parts *Mus rattus* is the common rat. Whereas *Mus norvegicus* succumbs readily to an ingestion of the broth culture as prepared by the Institute, we found the *Mus rattus* animals immune. Before we started for Java, we tried the pathogenic influence of the culture as furnished to farmers, on some of our cultivated rats of the *Mus rattus* group, on request of our ministry of colonial affairs. The rats were fed on a broth culture of a virulent strain of paratyphoid and bread, at the Serum-institute, and they remained in good health on this diet. The same culture killed practically all *Mus norvegicus* rats in a few days.

To our great regret we have never yet succeeded in obtaining hybrids between the two groups of rats, *norvegicus* and *rattus,* and for this reason the inheritance of this very marked immunity of *Mus rattus,* or in other words predisposition of *Mus norvegicus* can not be studied. We know of no case in the literature, of an investigation of the inheritance of immunity to bacterial disease in animals.

As is well known, Biffen found a case of the inheritance of resistance to rust in wheat, in which the difference between immune and easily infected plants was proved to be due to presence or absence of one single gene. William Orton and Webber have since found almost similar instances in cotton and watermelons.

So far as known to the authors, the following case of the inheritance of immunity, or predisposition for a microbial disease in animals is the first one studied so far.

From Nagasaki, Japan, and Hong Kong, China, we brought along some stock of a very minute domestic mouse. These mice evidently belong to the same group as the commonly imported oriental Waltzing mice. As a matter of fact, our Japanese animals of the second importation produced some waltzing offspring. We used this material for a few series of experiments on the inheritance of weight, one series starting from the only fertile Hong Kong female, and the others from diverse combinations of the Nagasaki strain with large white mice. These white mice are of a pure-bred strain used by T. B. Robertson in his experiments on growth. We produced numerous hybrids, great numbers of $F_2$ animals, and further we are grading back the hybrids both to the dwarf and to the heavy strain. For our work individual

mice are frequently weighed, and from time to time the whole series is weighed.

In the beginning of January an epidemic started in our mousery. Our mice were at that time housed in approximately seven hundred cages containing several thousand mice, both the size-inheritance and other series of breeding experiments. The cages of all the series were mixed and arranged on shelves in three adjacent rooms. The infection apparently swept through the entire colony, notwithstanding our attempts to limit it to one room. The Japanese mice were distributed over all the stacks in all three rooms, most of them mated to big mice or hybrids of different generations. All these mice fell victim to the epidemic, excepting three which we kept for a little while longer, by taking them into the living house at the beginning of the trouble. To our surprise the white mice of Robertson's strain proved immune. Even where the dead Japanese were partially eaten by their mates, these latter remained in good health.

It is clear that the main circumstance, which made it possible for us to see the clearcut segregation about to be described, was the rapid spread of the epidemic. All the Japanese mice were dead before the virulence of the microorganism was materially altered.

The rapid course of the disease made it possible to distinguish simply between dead and surviving mice. As a rule we found that animals contracting the disease presented the bunched up appearance and walked with the small, prancing steps familiar to students of paratyphoid in small rodents. They would be visibly ill for one, two, or exceptionally three days before death. We do not remember having seen one recover.

Professor Hall, of the department of bacteriology, of the University of California, was kind enough to make a bacteriological examination of the dying animals, and was able to isolate the same staphylococcus from the blood of the heart of four animals.

If we count the proportion of the animals which succumbed to the epidemic, we have to limit our countings to groups which are comparable. Immunity can never be anything but relative, and if we want simply to use the fact of survival as a criterion for immunity we must exclude as far as possible other causes of death. Of these the two main causes are death or illness of the mother, causing starvation of the young, and troubles in parturition.

In our study of the inheritance of immunity to this staphylo-

coccus infection we have therefore limited our counts to animals of the same age-group, that is to mice of at least four weeks old and not yet used for breeding.

At the general weighing of January 4, 1919, no losses were observed among the Japanese mice. Shortly afterward the Japanese started to die off. And at the general weighing of February 14, the last Japanese mouse was found dead.

The data given in this paper are taken from the records of this general weighing of February 14, 1919. They include litters of six kinds, pure Japanese, pure Robertson's whites, $F_1$ hybrids, $F_2$ hybrids, mice with one parent $F_1$ and the other Japanese, and such with one parent $F_1$ and one large parent.

As noted above all the Japanese left in the mousery died between January 4 and February 14, 59 in all. Of these 23 were in the class of weaned young, not yet breeding.

TABLE I

| Litters of $F_2$ Animals Jan 4 | Same Litters on Febr. 14 | Litters of $F_2$ Animals Jan 4 | Same Litters on Febr. 14 |
|---|---|---|---|
| 7 | 4 | 1 | 1 |
| 3 | 2 | 3 | 3 |
| 6 | 6 | 3 | 3 |
| 2 | 2 | 6 | 2 |
| 6 | 6 | 2 | 2 |
| 5 | 2 | 5 | 4 |
| 5 | 4 | 4 | 4 |
| 4 | 3 | 3 | 2 |
| 2 | 2 | 1 | 1 |
| 3 | 3 | 3 | 1 |
| 4 | 3 | 6 | 5 |
| 5 | 4 | 7 | 6 |
| 6 | 3 | 3 | 3 |
| 6 | 4 | 2 | 2 |
| 3 | 1 | 4 | 0 |
| 5 | 3 | Total 125 | 91 |

As to the Robertson large strain, no deaths were observed within this period of six weeks among mice of this age class. A very considerable number of these weaned young were growing up in cages together with Japanese of their age and sex.

Between January 4 and February 14 we lost no $F_1$ animals after weaning age. Strictly comparable to the other lots were only three litters, which were weaned within the critical six weeks and not yet put to breeding. These litters contained fourteen young. All were living on February 14.

This shows how the immunity to this staphylococcus disease of the large albino strain as opposed to the predisposition to it of the Japanese strain, is completely dominant in the hybrids.

To our great surprise we found that this difference between immunity and predisposition was caused by presence or absence of one single genetic factor. In other words, we found a very clear monofactorial Mendelian segregation in $F_2$. As we are weighing non-breeding $F_2$ animals up to a relatively high age, thirty-one litters containing 125 animals fell into this class between the two dates.

Of these 125 animals 91 were living on February 14, and 34 had died. (Theoretical expectation 93.75 : 31.25.) See Table I.

If in reality the "Robertson" mice have one gene, lacking in the Japanese, whose presence protects them against death from this infection, we would expect the hybrids to produce 50 per cent. gametes with and as many without this gene. As the Japanese lack this gene, we would expect 50 per cent. of the young from matings between $F_1$ and Japanese to be immune, and 50 per cent. to die. Fourteen such litters were available for the test, with 57 animals. Of these 57 on February 14, there were 25 left, 32 having died. (Theoretical expectation equality.) See Table II.

TABLE II

| Litters of $F_1$ × Japanese on Jan. 4 | Same Litters on Febr. 14 | Litters of $F_1$ × Japanese on Jan. 4 | Same Litters on Febr. 14 |
|---|---|---|---|
| 6 | 3 | 2 | 2 |
| 7 | 1 | 2 | 1 |
| 3 | 1 | 5 | 1 |
| 5 | 5 | 3 | 0 |
| 4 | 4 | 5 | 1 |
| 6 | 4 | 5 | 0 |
| 2 | 2 | 2 | 0 |
| | | Total 57 | 25 |

In the same class with the other litters we had sixteen litters of young, each from one $F_1$ and one "Robertson" parent. This gave us 51 mice in this class. Fifty of these were living February 14, one having died. (Theoretical expectation no deaths.)

As will be seen in nearly every case the number of deaths was slightly greater than expectation. Occasional mice will die even when given the best of care. It is indeed remarkable that not more of these vigorous mice, kept for the most part in company with several of their own sex, got killed fighting. It must

be remembered that these figures for deaths comprise all cases of absence. Mice killed in fights and animals escaped are classed as dead.

The numbers published in this note were collected only after the epidemic had done its worst, and from our weighing records. The epidemic seriously interfered with some of our planned series in our breeding work on weight.

It was planned to start a series of infection-experiments with the isolated staphylococcus strains on families of $F_2$ animals. It may be possible at some future date to do this, when the material will again be in the right condition for the experiment, that is to say, free of spontaneous infection. At present, however, it is evident that the staphylococcus infection is still in our mousery. The mortality in $F_2$ families remains high. It is clear that, if we subjected $F_2$ animals to infection with a pure culture of the staphylococcus, the group of animals would be already a selected group, and the results would be quite misleading.

We have refrained from publishing these data for some time, hoping that we could free our mousery from the infection, so that we could repeat under conditions of a laboratory experiment the immunity tests of $F_2$ families. There seems no further reason now to withhold the facts such as they are.

As far as we are aware no wholly comparable instance is known so far of a gene whose action has such a definite effect upon the resistance to a bacterial disease in animals. The evidence for the inheritance of a differential susceptibility to transplanted tumors in Japanese and large mice in the work of Tyzzer is scarcely as definite as our case.

In any case, this instance recorded here proves clearly that the presence of a definite pathogenic organism as a factor in a transmittable disease need not be the sole determining cause of the disease. And it shows that the search for heritable factors in the causation of bacterial diseases is neither hopeless nor unscientific. We can only hope that cases such as the one just given will encourage those medical investigators who believe that predisposition is a factor not to be lost sight of in the press of bacteriological and related discoveries.

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Berkeley, Cal.,
August 20, 1919

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## NOTE ON THE PHOTIC SENSITIVITY OF THE CHITONS[1]

1. The remarkable sensory organs discovered by Mosely (1885) in the tegmentum of the shell-valves of certain chitons are structurally of such a nature that in their most highly developed forms they were from the first recognized to be "eyes." Practically nothing has been made known as to the functional values of these organs, which in different genera occur in a great diversity of form, number, and arrangement. It has been shown, however, that the tegmental æsthetes of *Chiton tuberculatus* are indeed photosensitive (Arey and Crozier, 1919). But the shell-eyes are in this genus generally represented by structures of an intermediate degree of complexity. The "eyes" are supposed to have been derived from large, relatively undifferentiated shell receptors (macræsthetes), and seem to reach their highest development in those species of *Schizochiton* and *Tonica* which possess large complex eyes, each surrounded by a pigment cup (*cf.* Plate, 1899; Nowikoff, 1907, 1909); in *Chiton* (at least in some species of this genus) the eyes are "*intrapigmental,*" pigment being contained within the receptor cells, whereas with the "*extrapigmental*" eyes the associated pigment occurs outside

[1] Contributions from the Bermuda Biological Station for Research.

the receptor cells proper, in the integument. It seemed profitable to attempt an analysis of the functional values of the several types of photoreceptive elements to be found in different chitons. Accordingly, in 1918 I made observations on the photic irritability of representatives of several genera available at Bermuda. Pending the collection of more information on this subject, which is necessary for a full discussion of the problem here suggested, I give briefly the net result of these observations.

2. *Ischnochiton purpurascens*—found along the shores of islands in Great Sound, and in bays on the south shore of Bermuda, usually in more or less exposed situations, but commonly a little lower than the lowest reach of the tide (never between tidal limits)—is quite sensitive to light. Individuals about 1 cm. long were frequently obtained on bottles which had been on the bottom long enough to acquire a film of algal growth; the under surfaces of such bottles, and of smooth stones, provided most of my specimens. These animals were photonegative to light of any intensity used—from very weak diffuse light to direct sunlight. This species therefore resembles *I. magdalenensis* (Heath, 1899). It is said that among the *Ischnochitoninæ* there are no shell eyes. However, *Boreochiton*, also of this family, never occurs "far from the light" (Pelseneer, 1906, p. 50).

*I. purpurascens* is an active creeper (Crozier, 1919). It orients very quickly and precisely away from a source of illumination. At night its photic irritability seems decidedly enhanced, as I learned by comparing the rate of orientation of single individuals to lamp light, in a dark room, at different times during the twenty-four hours. (This would appear to be the case with *Chiton tuberculatus* also—*cf.* Arey and Crozier, 1919.) No evidence was had that *I. purpurascens* is reactive to changes of light intensity.

3. *Acanthochites spiculosus.* Specimens about 14 mm. long were found under stones, somewhat beneath low water level, in Ely's Harbor and at Spanish Point. In these places the water of the open ocean is less modified than within the sounds. The requirements of *Acanthochites* seems in this respect more rigorous than are those of the preceding species, for *A. spiculosus* was not found well within Great Sound. As in the case of *Ischnochiton*, the present species is decidedly photosensitive, and orients precisely away from the light. It moves faster away from a bright light than from a weak one, and comes to rest in the shade. It is strongly thigmotactic, tending to settle in the angles at the

corners of an aquarium, and once in such a situation is difficult to move by light. Negative geotropism is also fairly well pronounced.

If the intensity of light falling on an *Acanthochites* be suddenly increased, the girdle is depressed into contact with the substratum. Local illumination confined to the girdle leads to a local response of the same character. The shell plates seem not to be sensitive in this respect.

As in the case of most Chitons (Sampson, 1895; Crozier, 1919; Arey and Crozier, 1919), the body may be strongly curved to one side, the animal pivoting in a circle of short radius. Photic orientation is often accomplished in this way. The "pivoting" of *Acanthochites* ceases instantly when the creature is shaded; orientation is resumed when the light is increased. Since the girdle does not respond to shading of this part alone, I am led to believe that the shell plates are probably responsible for this type of reaction (as with *Chiton;* Arey and Crozier, 1919).

4. As elsewhere described (Arey and Crozier, 1919), the shell-plates of *Chiton tuberculatus* contain receptors activated by light of constant intensity and by shading.

5. An unidentified species of *Tonica,* about 6 mm. long, commonly obtained in company with *Ischnochiton purpurascens,* was found not to be reactive to shading, nor to increase of illumination; but, like the latter, was decidedly photonegative. This form is not so reactive to light as *Ischnochiton,* however.

6. Plate (1901) considered it possible that the order of evolution of the shell eyes of Chitons was from megalæsthetes to intrapigmental eyes to extrapigmental eyes. In the present series of species, this order would be represented by *Ischnochiton, Chiton,* and *Tonica,* in respective sequence. The shell eyes are of course not the only photoreceptors in these animals; for the girdle the ventral surfaces of the body (Arey and Crozier, 1919), and, possibly, the bilateral larval ocelli (Heath, 1904) are functional in this respect. But the experiments recorded in this paper show that functions of a certain diversity are served by the tegmental photoreceptors of the several species. Little can definitely be said, however, regarding the correlation of structural features with functional performance. It is noteworthy that members of the *Ischnochitoninæ*—a group characterized by the absence of shell "eyes" (*i.e.,* with megalæsthetes and micræsthetes only)—are quite as reactive to photic irritation as are members of *Chiton* proper, where, so long as the tegmentum is uneroded, eyes of the

intrapigmental type are functional; they are also more reactive than *Tonica* is, although in the latter extrapigmental eyes are developed. *Acanthochites*, moreover, likewise with intrapigmental "eyes," is reactive to shading, as in the case of *Chiton*, while *Tonica* is not. We are therefore unable to assign definite types of irritability to the several forms of shell photoreceptors.

The position taken by Nowikoff (1909), on morphological grounds, that these organs are not related in genetic sequence, is not inconsistent with such functional data as I possess. He regards the intra- and extrapigmental eyes as being independently derived from megalæsthete structures. It is possible to consider that the megalæsthetes (or certain of them) are activated by light, and that this kind of irritability is simply retained by eyes of the extrapigmental variety, whereas eyes of the intrapigmental sort are in addition activated by shading. However, the local activation of the girdle (of *Chiton*) by light and by shading makes it necessary to believe that tegmental mircæsthetes (*e.g.*, of the girdle scales) may also be implicated in the photic irritability of the shell-plates. As yet, experimental data for the analysis of this problem is incomplete. The possible significance of the number of shell-eyes present also needs to be investigated.

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## THE BIONOMICS OF PORICHTHYS NOTATUS GIRARD

*Porichthys notatus* is a batrachoidid fish, which is known to range from southern Alaska to the Gulf of California, and from depths of at least 62 fathoms to just above the lower low-water level of the reefs. During the fall and winter months it inhabits comparatively deep water, where nothing definite is known concerning its life, beyond the fact, recorded by Dr. and Mrs. Eigenmann (1889, p. 132), that it is at least occasionally preyed upon by rock-cods (*Sebastodes*). In the late spring and early summer a shoreward migration apparently takes place (Greene, 1899). Along the coasts of Lower California and on the mainland shore of southern California, it is usually found in shallow bays at this season; at Santa Catalina Island Holder (Holder and Jordan, 1909) has mentioned hearing numbers just off rocky shores. There is but one record of the occurrence of the species in the reef-pools south of the vicinity of Pt. Conception in California, Hilton (1914) having found a specimen in a pool on the reef at Laguna Beach, California.

From Pt. Conception northward, on the contrary, this species, while never abundant is by no means rare along the reefs within tidal limits, during the breeding season. Here it occupies very shallow, often sandy pools, either those containing boulders or those with horizontal crevices in the rocky sides. It is here a fish of sluggish and retiring habits, swimming slowly with an undulating motion; when disturbed it usually seeks shelter, but sometimes swims off a short distance only, coming to rest and

partially covering itself with sand by a lateral twisting of the body. It is able, by a sudden movement of the body, to inflict a rather painful wound with the opercular spine.

The stomachs of the specimens examined from reef-pools were mostly empty; one contained some sand and an empty snail shell, while another had eaten a crab (*Petrolisthes*), of a species which abounds beneath stones along the fore-shore. Eigenmann (1892) found an anchovy in the stomach of a *Porichthys* from San Diego Bay, and the writer found a sardine (*Sardinia cœrulea*) in the stomach of a specimen from San Diego County.

The adult is dull brownish, varying very little in color and not much in shade. The photophores are evident as silvery spots, due to the reflection of external light. There is a whitish translucent spot below the eye, and another behind the pectoral fin, in the position of a large pore in *Batrachus tau*. Owing perhaps to a greater development of black pigment, the males retain more of the dark pattern of the young than the females do. The coloration of specimens 26 mm. long was described in the field as follows. Eight greenish black bars extend from the mediodorsal line to the upper edge of a broad silvery stripe with metallic reflections, which occupies the middle third of the body. The fins are clear, excepting a basal caudal bar, and the two dorsal spines. The head is mottled with dark above, and is silvery on the sides and clear below, excepting the dark ring surrounding each photophore. A conspicuous narrow black streak, located below the eye, branches once or twice posteriorly.

Parental care is generally practised in the Batrachoididæ. *Porichthys notatus*, as noted above, after migrating shoreward during the late spring and early summer, breeds in shallow water, within tidal limits from the region of Pt. Conception northward, where all of the following observations were made. No details of the breeding habits prior to the guarding of the eggs have heretofore been published. Males with enlarged testes were taken by the writer on several occasions from June 2 to June 15, in no case guarding eggs, and in one instance, on June 20, one was found in company with a female containing matured eggs. A male with ripe testes was found washed up on the beach near the reef of Government Pt., near Pt. Conception, on July 15. The females must leave the pools as soon as, or soon after, the eggs are laid, as none other than the one just mentioned was observed in the tidal zone. As Greene (1899) has already remarked, it is the males which guard the eggs and

young, remaining within a few feet of them even when disturbed. The eggs are cemented to the roof overlying a shallow crevice in the rocks or a space beneath a flat boulder. They somewhat suggest the familiar egg of the Pacific salmon in color, and vary in the larger diameter from 4.0 to 6.0 mm. They are slightly compressed, as though by pressure against the rock, and are broadly elliptical in outline.

The young hatch out during the summer. Jordan and Starks (1895, p. 840), in discussing the species as found in Puget Sound, remark "the young fasten themselves to the rocks by means of a ventral disc which soon disappears." They mention further that "the adult remains with the young until they are quite well matured." On October 25 the writer found a single grunting male under a large flat stone in a pool about two feet square, with numerous young all about 26 mm. long. Other young, 22 to 28 mm. long, were caught in a larger pool on October 26. None has been obtained on the reefs in the winter or spring; young as small as 23 mm. have been taken by the Scripps Institution for Biological Research, at La Jolla in depths as great as forty fathoms. Except for their proximity to the eggs, the males show no special habits which might be construed as definitely protective.

*Porichthys* is one of three genera of phosphorescent shorefishes, the other two being *Anomalops* and *Photoblepharon* of the East Indian reefs. In each of these East Indian fishes the single large light-producing structure is located below the eye (Steche, 1909), while in *Porichthys* a large number of photophores (in *P. notatus* Greene found an average of about 700) are developed in connection with the several lateral lines (except the uppermost, which is only rarely accompanied by a few rudimentary light organs), one photophore being opposite each pore. The photophores are most abundantly developed on the ventral surface, and all are oriented downward. The same condition holds true in the several other diverse groups of fishes, mostly pelagic or bathypelagic, in which the power to emit light has obviously been independently acquired, as well as in certain other phosphorescent animals, such as the bathybial cephalopods. This general downward cast of the light of luminescent marine animals, a point regarded by the writer as of critical significance, has apparently not been duly considered by any of the authors who have proposed such varied theories to explain the biological significance of biophotogenesis.

The histology of the photophores of *Porichthys notatus* has been reported upon in detail by Greene (1899), and ten years earlier by Eigenmann and Eigenmann (1889), and by Test (1889). These organs were found by the writer to be completely and typically developed in young about 25 mm. long (sectioned at Stanford University). Each photophore lies deeply imbedded in the dermis. It consists essentially of a more or less parabolic reflector surrounded by a mass of melanophores, and enclosing the photogenic gland cells, which are richly supplied with blood vessels, but according to Greene not specifically enervated. The light passes downward either directly from these cells, or indirectly from the reflector, through the large lens and the cornea-like epidermal covering of the organ. The ventral aspect of the illuminated fish is striking, the hundreds of dots of light being arranged in a symmetrical pattern.

The light of *Porichthys* has been observed only in the laboratory, where it has been produced as a response to intense electrical or chemical stimulus. Green records but one instance of a slight glow of the photophores being produced by mechanical stimulus. In experimenting with two specimens from rather deep-water, the same author was unable to produce any reaction of the photophores, although he applied stimuli successful in the case of individuals from the reefs. This observation, while of course insufficient, perhaps indicates that the luminescence may be characteristic of the breeding season. In this connection it is also significant to note that Holder's observations (Holder and Jordan, 1909) indicate that the species is of nocturnal habits, its grunting being heard chiefly at night, or in the evening or early morning.

The peculiar humming sound produced by this species during the night, or during the day when disturbed, is another of its remarkable characteristics. The sound (which by some has been called a "song," an expression which seems quite figurative) is produced in the air-bladder, which has a very thick muscular wall, and is enervated by the thickest ramus of each vagus nerve. Upon opening a live specimen the air-bladder was observed by the writer to vibrate rapidly while the fish was grunting, and the sides of the body were felt vibrating at the same time. The abdomen of young about 25 mm. long was also felt vibrating, but the sound produced, if any, was inaudible.

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# STENOTHERMY AND ZONE-INVASION[1]

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The conception of geographical distribution seems to have come to the botanists of the later fifteenth and earlier portions of the sixteenth centuries as a distinct and gradually developing idea. They began to realize that the plants of central and northern Europe were different from those of Greece and Italy as treated of by Theophrastus and his successors and were worthy of study for their own sakes. With the revival of learning, the discovery of the "New World" and the attention paid to the plants and animals of the different countries being made known through the visits of the various voyagers, both the knowledge of the different countries and that of the natural objects brought back from them emphasized more and more the idea of geographical differences in flora and fauna and the gradual perception that there might be some general laws or principles governing them. It remained for Humboldt in a series of papers in 1805, 1807, 1816, 1817 and 1820, to place the matter of the geographical distribution of plants on a firm scientific basis. After Humboldt's preliminary work, came the studies of a number of leading botanists and gradually there have arisen various points of view, especially as to factors concerned and as to the division of the subject into various categories according to the special factor, or set of factors, emphasized. The studies in ecology, which have come more recently to represent the activities

[1] Annual lecture before the Barnard Botanical Club, New York City, delivered March 12, 1920.

toward solutions of the problems of distribution, are founded more particularly on the influence and control of distribution by edaphic factors, or those more particularly connected with the substratum and concerned in the studies of association and formations, in other words having to do particularly with topographic distribution as contrasted with climatic distribution.

Humboldt's publications concerned themselves particularly with climatic distribution, although, incidentally, he necessarily touched upon topographical distribution. The chief factor of control in climatic distribution recognized by Humboldt and his successors is temperature, and Humboldt called attention, in most graphic ways, to the resemblances between the climatic zones of latitude over the earth's surface and those of altitude passed through in the ascent from sea-level to thousands of meters above it. Those of us who are older remember the reproductions of Humboldt's diagrams of the various zones (or perhaps better, belts) of vegetation of mountain peaks situate in different latitudinal zones which were reproduced in the various atlases and older geographies. Lamouroux, in 1825 and 1826, applied the general principles of Humboldt, DeCandolle and Robert Brown, to marine plants, especially to the algæ, and distinguished latitudinal zones and differences of distribution in depth (belts), as well as the effect of certain factors on topographical distribution. Lamouroux was followed by Greville and Harvey in the attempts to discuss the distribution of marine algæ and the latter (1852) divided the Atlantic coast of North America into 4 divisions and emphasized the position of Cape Cod as a demarcation point. These authors and their ideas may be taken as starting points of the discussions more or less contemporaneous and later.

A definite attempt to determine the criteria of a climatic zone was made by C. Hart Merriam (1894, 1898) in his papers on "life-zones" and "crop-zones" of the United States. Merriam used summation indices compiled for a large number of stations and divided the

country into life-zones in accordance with the indices thus obtained. Merriam also shows that, by plotting the isotherms of 18°, 22°, and 26° C., for the six hottest weeks of the year, divisions are separated from one another corresponding in all essential details to those obtained by plotting summation lines. This is practically the method I have used in separating climatic zones of the surface waters of the oceans. Livingston and Livingston (1913) have discussed the system of Merriam and proposed a system of efficiency temperature coefficients which are claimed to represent something more of the basic principles of physiology upon which the final explanations of distribution should be based. A comparison between isoclimatic lines plotted for the United States on the direct summation basis and isoclimatic lines plotted on the efficiency indices basis shows a strong, but not absolute tendency toward agreement. The Livingstons, however, do not discuss the interpretation of their charts as regards plant distribution in detail.

Nearly 30 years ago, while attempting to obtain some idea of the temperature relations of the geographical distribution of the Laminariaceæ, I noticed a seemingly definite relation to the lines of mean maxima (summer lines or isotheres) of surface temperatures. Some brief remarks on these relations were published in 1893. Farther studies seemed to emphasize the relation between the 10, 15, 20, and 25 degree (Centigrade) isotheres or lines of mean monthly maxima and the limits of distribution of various floral groups, and in 1914 I read a paper at the Twenty-fifth Anniversary Celebration of the Missouri Botanical Garden (published 1915) as a preliminary communication on the temperature relation of the distribution of the marine algæ as expressed in terms of mean monthly maxima and minima (isotheres and isocrymes). In this paper, I made a tentative division of the surface waters of the oceans, etc., into zones according to the courses of the 10°, 15°, 20°, and 25° C. isotheres, and announced that a rough tabulation indicated that the great majority of species are confined to one or

another of these zones, that a considerable number of species extend over two of these zones, that a comparatively small number are found to extend over three zones, while the number credited with extending over four or five zones are extremely few and almost always doubtfully so accredited. It was also suggested that the disturbance of zonal distribution, so far as the occurrence is concerned, is probably due to spot distribution, *i.e.*, where waters of a higher or lower temperature than that of the zone in which they are placed exist due to local physical conditions, and to seasonal lowering of the temperature normal to the zone. In 1916, in another address (published 1917), I reasserted these statements and added something as to the significance of the isocrymal lines, or lines of monthly mean minima. I suggest using the latter lines to divide the zones into proper provinces.

Since writing the last paper I have investigated the floras of the coast of New England and have found that the species may be readily arranged in two categories, one of the colder waters (20° C. or less) and the other of the warmer waters (20° C. or over), and while some of these are found only north of Cape Cod and others only south of Cape Cod, the majority are found on both sides of the cape, which is, however, the natural dividing point and approximating closely to the position of the 20° C. isothere. The separation is made by ascertaining whether a given species of the last group in particular inhabits warmer localities to the north or is found only in cold localities or appears or fruits only in the colder season to the south. Similar examinations of other, but less perfectly known floras, add to the conviction that species of marine algæ, at least, are normal to only one zone of 5° C. amplitude as to mean maxima, except in the cases of those of the very coldest waters and it may be that they are no exceptions to such a rule. Furthermore, it may be assumed, from observing the isotheres and isocrymes in favorable portions of the surface waters of the oceans, viz., those undisturbed by the larger ocean

currents, that the normal, or at least the minimum seasonal variation in temperature is closely approximating to 5° C. This, added to the amplitude of 5° C. mean maximum variation, makes the normal amplitude of temperature within each zone about 10° C. and the temperature interval favorable to the persistence of a species within a given area, so far as active growth is concerned, is very little, if any, over 10° C. The seasonal range in some portions of the surface waters of the oceans may amount to as much as 18° or 20° C. In such localities as may have such an extreme range of temperature, we may, I think, assume that a condition of quiescence, or rigor, may exist, at least in the perennial species, such as exists in the case of perennial plants in zones on land where there is an alternation of a frost with a frostless season, as it does particularly in the polar and most of the so-called temperate regions.

It will appear from a careful consideration of what I have been saying, that the temperatures for normal persistence of any particular species of marine plant lie within narrow limits, although many marine plants are credited with extending over fairly wide ranges of temperature. We are, consequently, brought directly to a consideration of the ideas implied in the use of the terms stenothermal and eurythermal. The proposal of these terms, or rather their equivalents in German, rests with Karl Moebius who, in 1877, published a paper in *Die Natur* on the external factors of life of marine animals. According to Moebius the eurythermal animals can endure wide ranges of temperature and continue their occupation of extensive zones and range in depth because they are able to reproduce under such conditions. It is this conception of being able to reproduce at widely separated temperature limits that I wish to call particular attention in order that I may discuss it later. Moebius states that the eurythermal animals are much less numerous than those which normally occur and withstand a narrow range of temperature which he calls stenothermal animals. The latter seem to be the more

usual type and this agrees with what I have found in my attempts to tabulate the marine algæ as I have already mentioned with emphasis. To repeat the idea of Moebius, in a rather free translation of his own words, eurythermal animals are those which in the surface waters of the temperate zones are able to exist and to continue their kind through reproduction under all of the various temperature relationships of the different seasons of the year.

The terms eurythermal and stenothermal have not come into any noticeable use in botany and are not widespread even in zoological literature, although they are very convenient. They are both to be found in the later editions of Webster's and in the supplement to the Century Dictionary. They are discussed in the latest edition of the Encyclopedia Britannica by G. H. Fowler, under the article on "Plankton." Fowler says: "In relation to temperature the wide-ranging species are termed eurythermal, the limited stenothermal (Moebius); the terms are useful to record fact, but not explanatory. It seems to be the case that to every organism is assigned a minimum temperature below which it dies, a maximum temperature above which it dies and an optimum temperature at which it thrives best; but these have to be studied separately for every species." The definitions of Moebius and the comments of Fowler are exactly to the purpose of our consideration, since the one is from the purely distributional point of view expressing a fact only, while the other seeks to link the fact with some explanation, preferably physiological. Our own discussion of these terms and the underlying conceptions must necessarily proceed on a somewhat middle course, largely from the distributional point of view, but with such regard for the interpretation of the physiological basis as may be possible from our present knowledge.

It will be of the greatest assistance, I think, to consider some concrete cases of eurythermal species and to inquire into the conditions of their continued persistence

under different temperatures. One constituent of the marine flora of the northern hemisphere which has interested me very much indeed, is the common eel-grass, *Zostera marina,* a marine spermatophyte. As commonly regarded as to specific limits, this plant extends from the northern coasts of Europe down along the western coast and enters the Mediterranean Sea, occurring spotwise in the northwestern portion of it and being represented also in the northern Adriatic. *Zostera marina* is represented in one or two localities in southwestern Greenland and, reappearing at the Strait of Belleisle, it seems fairly continuous in its distribution thence down to the coast of North Carolina, at least, and is reported from West Florida and the Bermuda Islands, although I can not make certain as to whether it actually grows in either of the last mentioned stations. *Zostera marina* is reported from both the North American and the Asiatic coasts of the North Pacific, but the exact limits of its occupancy of these shores is in doubt. The greatest range of temperature experienced by the *Zostera* is that on the Atlantic coast of North America, where it extends from waters of a mean maximum of 0° C. to those of a mean maximum of somewhat over 25° C. It is also found in localities where the seasonal temperature range of the surface waters is from somewhat below 0° C. to 15° C. It ranges through all the temperature zones of surface waters from the Upper Boreal (0°–10° C.) to the Tropical (25°–30° C.), *i.e.,* five zones in all. I shall discuss the reasons for this wide extension of the range of *Zostera marina* later, but desire to call attention here to the facts that we are dealing with a perennial plant with unusually effective methods of vegetative multiplication and devices for wide dispersal.

Another eurythermal marine species is *Ascophyllum nodosum,* one of the bladder-bearing Fucaceæ or Rockweeds. On the Atlantic coast of North America, this species is found in some abundance from well up on the west coast of Greenland down to the northeastern coast of New Jersey, or on coasts having a range of mean

maximum temperature from 0° C. or below to about 22° C. and seasonal ranges of about 17° C. maximum. *Ascophyllum nodosum* is also a perennial species. *Rhodochorton Rothii* is a delicate red alga which, nevertheless, seems to be a perennial and *Monostroma Grevillei* a membranous green alga, and *Polysiphonia urceolata,* a filamentous red alga, are annuals, but with the same range as *Ascophyllum nodosum. Grinnellia americana* is the last example of many eurythermal algæ of the Atlantic coast of North America I desire to bring forward. It is a strikingly beautiful annual membranous red alga and extends from northern New England (North Temperate Zone, 15°–20° C., mean max.) to the coast of North Carolina (Tropical Zone, 25°–30° C., mean max.). Other examples of eurythermal species might be given, but those I have mentioned are typical and reasonably well known. They will serve as a good representative basis for discussion with the idea in mind that what is indicated by the eurythermy of one and another of them will, by analogy, also seem extremely possible to be the case with all other types and individual species extending over ranges of temperature of more than 10° C.

Stenothermal species are particularly characteristic of the Tropical Zone, in very few portions of which the seasonal variation in temperature is over 10° C. Species confined to the Upper Boreal or to the Upper Austral Zones are also narrowly stenothermal, since the entire range of temperature in these zones is not over 10° C. The temperate and subtropical zones are usually sufficiently affected by seasonal changes to show a range of temperature greater than 10° C., but in the southern hemisphere in particular, there are portions of these zones, at least, that show only a 10° C. range and consequently may possess stenothermal species. The annual species of any particular zone, and even perhaps all annual species, are stenothermal so far as their actively vital processes are concerned, but may endure temperatures of more extended range in the resting seed or spore condition. This naturally brings us to inquire as to the

nature of the fundamental differences between the eurythermal and the stenothermal species, and this, in turn, is closely connected, as I shall hope to make plain, with the second topic of this paper, viz., zone-invasion.

I have already tried to make clear the fact that my investigations have tended very strongly to convince me that each and every species of marine plant is normal to only one zone, and that, when a species is credited to, or found to occur in, two zones, it is normal to only one of them and is to be found in the other because for some reason it finds in the second zone the temperature conditions, both as to degree of temperature and as to duration of that degree of temperature, of the zone to which it is normal. In a similar fashion, if a species is found to inhabit three, four, or even five zones of different temperature relations, it is possible to make certain that it is normal to only one of these and invades the other zones because it finds the proper temperature conditions for its continuous existence. The proper temperature seems certainly to be that which is most intimately connected with reproduction, since it is this function that is most necessary to persistence in the particular locality. In the laboratory, under controlled conditions, algæ, in particular, have been found to be very sensitive to even slight changes of temperature, as Ewart (1896) has demonstrated. It does not seem as if the same algæ, in their ordinary environment, could be thus sensitive as West and West (1898) have held, but Ewart (1898) has answered their objections, claiming that, in nature, they are probably equally sensitive, but withstand seemingly great changes for reasons that prevent these changes actuating. This is something of the truth in the case of species invading colder from warmer, or warmer from colder zones in that they find in the invaded zones the temperatures, both as to intensity and duration, which are favorable to their growth and reproduction and which are the same as they find normally in their proper zone.

Zone-invasions proceed in one of two directions, or,

occasionally, in both. They may proceed from warmer to colder zones, they may proceed from colder to warmer zones, or they may proceed from a zone of intermediate temperatures to both colder and warmer zones. Where warmer spots or areas exist in the midst of cooler waters, species of warmer zones may exist spotwise in the cooler zone. Where certain portions of the waters of a warmer zone are depressed in temperature by cold currents or upwellings, or, for certain seasons of the year, suffer a general lowering of the temperature, there and then may species from cooler zones be expected to put in an appearance. The extent of such invasions will depend naturally upon the intensity and duration of the unusual temperature. A consideration of the examples I mentioned as typically eurythermal may serve to make this idea more clear.

*Zostera marina* seems, on careful study of its occurrence and habits on all the coasts where it is found, to be normal to the North Temperate Zone with the mean maxima for the hottest month from 15° to 20° C. If this is the case, we are dealing with a species which extends in both directions from its normal zone. The more northern extensions may be explained by the fact that the very shallow and protected lagoons and interiors of prolonged and narrow bays preferred by this species may have the temperature of their waters raised through the action of the air and of the sun. In such waters, insolation undoubtedly is the most effective agent in raising the temperature as much as 10°–12° C. or even higher. To the south, the invasions of *Zostera marina* may be assumed to be made possible by the seasonal lowering of the temperature of the waters through the lower winter temperatures, *e.g.*, the winter temperatures on the coast of North Carolina is somewhat under 20° C. and the winter temperature on the coast of West Florida is also somewhat under 20° C. It would be expected, if the seasonal lowering of the waters south of the lower limits of the North Temperate Zone allows the eel-grass to find its normal temperature for fruiting in an earlier

season of the year than late summer, that the farther south the species grows, the earlier will be the fruiting season. Unfortunately, it is impossible to obtain any extensive data on this subject, but reliable testimony indicates that it flowers and fruits somewhat over a month earlier on the coast of New Jersey than it does on the coast of northern New England. It seems therefore that the critical temperature for persistence of this species, at least through flowering and seeding, is the same throughout its limits and that the species does not differ from a typical stenothermal species from this point of view. *Zostera marina,* however, is one of the most typical of the eurythermal species in that it must endure extremes of both heat and cold in various portions of its extensive range and in the various seasons of the year in each and every portion of its habitat. It is not known as to the temperature limits of the vital activity of the vegetative portions of the *Zostera,* but it does not seem possible that their separation can possibly be as wide as the differences between the extreme limits of the temperatures of endurance and probably are very much less. The *Zostera* probably has rather narrow limits to the temperature range of its vegetative activities and undoubtedly passes into a resting or hibernating condition, a condition of cold-rigor or of heat-rigor as the case may be, at the upper as well as at the lower portions of its temperature range. The land perennials of temperate zones do this and it seems safe to assume that *Zostera* does the same.

The case of *Ascophyllum nodosum,* a perennial brown alga of complex structure, is a very excellent one for study. This species ranges from the western coast of Greenland to that of New Jersey and it has a similar range on the northern and western European coast. On the coast of Greenland, it fruits in summer and it fruits earlier and earlier in the season as it proceeds towards the south, until, in the region of Long Island Sound, it fruits in late winter and early spring. The frond, or

vegetative portion, of *Ascophyllum* does not seem at all vigorous during the summer of the southern portion of its range. It seems perfectly evident that *Ascophyllum* is normal to the Upper Boreal Zone and invades the zones to the south because it finds, even on the north-eastern coast of New Jersey, seasonal temperatures of proper duration below 10° C. The isocryme, or winter isotherm, of 5° C. touches the coast of New Jersey at about the point that marks the southern limit of the range of *Ascophyllum nodosum.* We have in this species, then, a eurythermal species whose critical temperature and amplitude for persistence range from 0° to 10° C. and which undoubtedly passes into a condition of heat rigor during the hotter months of the year in the southern portion of its range. It differs from the last example, in that its course of invasion is in one direction, viz., to the south.

*Rhodochorton Rothii* is a very delicate, filamentous, perennial red alga of very lowly stature. It has a range very similar to that of the last species and, in the southern portions of its range, fruits only in winter. The same things may be said of this species as were said of *Ascophyllum. Rhodochorton,* however, is a shade or cave plant in the more southern portions of its range, seeking the cooler portions of the warmer districts. This is doubtless its only opportunity of surviving the heat and is of great benefit to its delicate structure.

*Monostroma Grevillei* and *Polysiphonia urceolata* are annuals, with about the same range as the last two. They are summer annuals in the waters of Greenland, but are winter and early spring annuals of the southern portions of their range. With the exception, then, of the temperatures endured by their resting spores, they are confined to the temperature range of the Upper Boreal Zone and are practically stenothermal.

The last example quoted is *Grinnelia americana,* an annual red alga, apparently normal to the Long Island Sound district and therefore of the North Subtropical

Zone (20°–25° C.). North of Cape Cod, it is to be found only in certain warm protected spots where the insolation is sufficient to raise the temperature to that of the subtropical zones while the waters outside are those of the temperate zones. To the south of the North Subtropical Zone the species is a winter annual and follows the 20° C. isocryme. *Grinnelia* is, therefore, a stenothermal and not a typical eurythermal species and invades both colder and warmer zones, the colder because of warm spots and the warmer because of favorable seasonal conditions.

Farlow, in his Marine Algæ of New England, has amply explained still a different type of invasion, viz., from a colder zone into a warmer and I have some additional details in a paper soon to be published. The *Laminariaceæ*, or kelps, a number of species of perennial red algæ, some other browns, greens, reds, etc., pass Cape Cod and are to be found in the colder waters which are usually the deeper waters to the south of it. This seems to be an invasion from the North Temperate into the North Subtropical. It does not mean, however, as is the case also with the examples I have mentioned and discussed, that these seeming invaders are living in waters of a different range of temperature from that of the normal zone. Their eurythermy is but seeming, at least so far as this particular invasion is concerned.

In conclusion, I may say simply this: stenothermy is the rule both from the point of view of distribution and of physiology, at least so far as effective reproduction is concerned; eurythermy is largely, if not entirely, a matter of endurance of a wide range of temperature, much of which endurance is due to the power to enter into a condition of rigor after certain extremes of temperature of either direction are passed; and a study of the various reasons for zone invasion assists greatly in making these facts apparent.

# PHYLOGENY OF THE ARTHROPODA WITH ESPECIAL REFERENCE TO THE TRILOBITES[1]

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THE phylogeny of the Arthropoda has been discussed so often that merely to summarize previous opinions would require an article of considerable length. In a recent number of the NATURALIST,[2] Professor Crampton has reviewed the subject from the standpoint of a student of insects, the most specialized Arthropoda. It may be of interest to see what results are reached when approached from the point of view of a student of the trilobites, the most ancient members of the phylum. The characteristics of trilobites may be summarized as follows:

## APPENDAGES

During the last two years I have had occasion to restudy practically all of the known specimens of trilobites whose appendages are preserved. The limbs of twelve species, representing nine genera, are now more or less fully known. Those which leave least to be desired are *Neolenus* from the Middle Cambrian, *Triarthrus, Calymene, Ceraurus,* and *Cryptolithus* from the Middle Ordovician, and *Isotelus* from the Upper Ordovician. Representatives of all three of the orders into which the class is divided are included in this list, which contains examples of both "primitive" and "specialized" trilobites.

The appendages of all these genera, with the exception of *Isotelus,* whose exopodites are still unknown, prove to be constructed on one plan. An articulatory segment

[1] This is an abstract of a more extensive discussion of the affinities of the trilobites now being published by the Connecticut Academy of Arts and Sciences.

[2] AMERICAN NATURALIST, Vol. 52, 1919, p. 143.

(coxopodite) supports the proximal ends of two branches, an ambulatory endopodite and a setiferous respiratory exopodite. The endopodite consists in all cases of six segments, the terminal one with movable spines on the distal end, usually three in number, but occasionally several. The proximal segment of the endopodite is a basipodite and gives rise to the exopodite, although both branches articulate with the coxopodite. The method of articulation of coxopodite, exopodite, and basipodite is similar to that of the second thoracic limb of the recent *Anaspides,* as figured by Calman. The exopodite is in all cases composed of a flattened shaft, along the posterior margin of which are delicate flattened setæ. The form of articulation of the basipodite, exopodite, and coxopodite indicates that when one of the outer branches moved the other accompanied it, but as the exopodites were always above the endopodites, they appear to have been of comparatively little use in swimming, and were probably chiefly respiratory organs.

All of the trilobites mentioned, so far as their state of preservation will allow determination, have four pairs of limbs of this sort on the cephalon, a pair on each segment of the thorax, and as many pairs on the pygidial shield as there are annulations on its axial lobe. In front of the biramous limbs there is one pair of uniramous, richly segmented, tactile antennules. The ventral membrane of the trilobite was very thin and feebly supported, so that the articulation of the limbs was not with it, but with infoldings of the dorsal shell which extended downward beneath the glabeller and dorsal furrows. The distal end of each of these appendifers fitted into a notch in the upper side of the corresponding coxopodite. A projection of this latter segment extended mesally nearly to the median line, forming endobases which on the cephalon, and usually along the whole of the body, functioned as food-getting organs.

No other parts of the limbs have yet been found, although I have searched diligently through all of the

known material preserving the ventral anatomy. Therefore it seems to me that Walcott has not sufficient evidence for the structures he illustrates and describes as epipodites and exites in *Neolenus*, epipodites in *Triarthrus, Calymene,* and *Ceraurus,* and spiral gills in the last two.[3] The presence of none of these things can, in my view, be proved.

There is little modification of the appendages of different parts of the body. The gnathobases of the coxopodites on the cephalon of *Triarthrus* are more jaw-like than those on the remainder of the body, and in the same species the segments of the endopodites of the pygidium and posterior part of the thorax are more triangular than those of the anterior ones. *Cryptolithus* has the thoracic legs bowed backward to form more efficient pushing organs, and in all species with long hypostomata the anterior biramous appendages seem to be more or less degenerate. Thus, *Calymene* appears to have two pairs of very delicate biramous appendages back of the antennules, and the first one or two pairs of gnathobases of *Calymene, Ceraurus* and *Neolenus* seem to be somewhat reduced, but there is no evidence that any pair of appendages is entirely lost. All the evidence seems to indicate that Beecher correctly homologized the cephalic appendages with the antennules, antennæ, mandibles, maxillulæ, and maxillæ of the Crustacea.

## FORM OF BODY

Trilobites are always depressed, flattened animals, with a broad head composed of at least five fused segments, a thorax of from two to forty-four free segments, and a pygidium made up of a variable number of undifferentiated segments. The anal opening is at the posterior end of the pygidium, and the growing point just in front of it, as in other arthropods. New segments are introduced into the posterior end of the pygidium during moults, are pushed forward by the introduction of others

[3] Smithsonian Miscl. Coll., 1918, Vol. 67, No. 4.

behind, and eventually a certain number are freed from the anterior end of the pygidium to form the thorax. Trilobites with an elongate worm-like form have numerous thoracic segments and small pygidia, while many others have few free segments and the pygidium nearly as large as the cephalon. These latter have usually been called more specialized than the former, but it is obvious from the method of introduction of new thoracic segments that the reverse is the case. This opinion is confirmed by a study of the ontogeny, for it is found that in the protaspis the pygidium of any species is proportionally larger than at any later period in life, and that many species pass through a stage in which they are isopygous.

There is a certain amount of evidence that the pygidium was used as a swimming fin, and some species seem to have had sufficiently strong muscles to enable the animal to dart away suddenly when attacked. Trilobites with large pygidia would thus have a certain advantage over the others, and, as a matter of fact, it was this type which persisted longest. The broad depressed body was not as well adapted for a nectic mode of life as a compressed fishlike one would have been, but it is the form which could most easily be kept afloat and propelled with the minimum of effort. The young of all species are circular or broadly oval in outline, and those adults with subequal shields depart least from that form. A fair inference from the above would be that the elongate crawling trilobite was more specialized than the isopygous swimming one.

## Internal Anatomy

Naturally knowledge of the internal anatomy is not as full as could be desired, but what follows appears to be based on reasonably clear evidence.

The mouth is ventral and usually situated back of the middle of the cephalon. Its position depends upon the length of the "upper lip" or hypostoma, and in extreme cases may be at the posterior end of the cephalon. There

is evidence from ontogeny and phylogeny of a backward migration of the mouth, coincident with the same movement of the eyes. In both cases this is probably due to the enlargement of the anterior end of the mid-gut. From the mouth the esophagus extends upward and forward to the enlarged mesenteron which occupies the greater part of the large cavity between the hypostoma and glabella. The intestinal canal tapers backward, but no differentiation of the posterior portion has yet been made out. The anus is beneath the posterior end of the axial lobe. The heart is elongate, chambered, branchiopod-like, and in the one species in which it is preserved, extended from the middle of the cephalon to the anterior end of the pygidium. The principal muscles were a dorsal pair of extensors, attached at the posterior margin of the cephalon and anterior ring of the pygidium, and a ventral pair of flexors, both with branches inserted in each segment. All of these organs were within the axial lobe.

### Comparison with Other Arthropoda

Having thus briefly stated the principal characteristics of the trilobites, the method will be to indicate briefly the similarities which exist between the trilobites and other arthropods, and to show that there is nothing about the bodily form or characteristics of the appendages to negate the possibility of a derivation directly or indirectly of all other classes from that under discussion. It is obvious that in this short paper each class can be treated but briefly.

### Crustacea

The trilobites are themselves crustaceans, as is amply proved by their biramous appendages. An attempt will be made to show that they may have been ancestral to the other crustaceans.

In recent years it has been generally considered that the Branchiopoda were more nearly allied to the trilobites than any other living animals. Bernard, the chief

proponent of this association, did not consider either subclass derivable from the other. Walcott has more recently stated that the trilobites were derived from the branchiopods and in this has been followed by Crampton. The points of relationship are: in both subclasses the number of segments is not fixed, and in both there are some species which have large numbers of them; both have a well-developed labrum (hypostoma); both have functional gnathobases along the body; the change in metamorphosis of the branchiopod is comparatively small, although *Apus* is by means a "grown up nauplius," as Bernard put it.

So far as these similarities are important, they do show a close relationship of the two groups, but none of them indicates that either is more primitive than the other. When a closer comparison is made, it at once becomes evident that the trilobites are much more primitive than the branchiopods. For example: trilobites have no carapace; some branchiopods do; trilobites have serially similar appendages on all segments, branchiopods have very different appendages on the head from those on the thorax, and some of the abdominal segments lack them entirely; trilobites have antennæ like the other cephalic and trunk appendages, branchiopods have the antennæ highly modified, degenerate, or absent. In other words, branchiopods are in all these respects much more specialized than the trilobites. Finally, the limbs may be considered. Lankester has shown that the schizopodal limb of the higher Crustacea may be explained as derived from one like that of the thorax of *Apus,* and most students of the Crustacea have followed him in considering the phyllopodous limb the most primitive among the Crustacea. This theory has now been completely upset, for Walcott has found several undoubted branchiopods with appendages in the Middle Cambrian, and the best preserved of them (*Burgessia*) show that the limbs were not phyllopodan, but like those of trilobites. The ancient branchiopods having had simple trilobite-like limbs, it

can no longer be held that phyllopodan limbs are primitive, and, stripped of their trilobite-like disguise, these wormlike crustaceans may no longer be considered most primitive. The possession of biramous limbs by the branchiopods of the Middle Cambrian, added to their other undoubted likenesses, indicates the possibility that they were derived from the trilobites, although some of them had then already attained the specialized carapace, pedunculate eyes, and limbless hind-body. This possibility is converted into strong probability when one considers the structure of the beautiful *Marrella splendens* Walcott. The head of this "lace-crab" of the Middle Cambrian is obviously highly specialized, but the structure as a whole proves it to occupy an intermediate position between the trilobites and more specialized crustaceans, including the branchiopods. It resembles the higher crustaceans in having the antennæ uniramous, in lacking exopodites on the cephalic appendages, gnathobases on those of the thorax, and in the absence of pleural lobes from the test of the trunk. This animal retains enough characteristics of the trilobites to show that it was derived from them, and has attained enough characteristics of the higher Crustacea to show that it belongs with them. A better connecting link can hardly be expected.

The Copepoda prove, on analysis, to be much more closely allied to the trilobites than had been supposed. All students have remarked upon the many primitive features of the non-parasitic members of this group, but have generally explained them by the sweeping assertion that they must be degenerate. Why, if they are degenrate, do the Copepoda show fewer modifications during development than any other Crustacea except the trilobites? They, instead of *Apus,* represent the "grown up nauplius."

These animals resemble the trilobites in lacking a carapace, in possessing pleural lobes, which, however, are incurved instead of being flattened. The greatest resem-

blances are, however, in the appendages. All these, except the antennules, maxillæ, and maxillipeds, are biramous, the antennæ and mandibles being especially like those of trilobites. The Copepoda are easily derivable from the latter, even though there are no fossil forms to connect the two groups. Since they lack compound eyes, and show very slight evidence of having ever possessed them, it is even conceivable that they branched off from the Hypoparia, the most primitive of trilobites.

The Ostracoda and Cirripedia are of course highly modified by their somewhat peculiar method of life, but when the young are studied, the characteristics of trilobites are readily observed. In fact, it is really surprising that the trilobite-like character of the crustacean nauplius is so consistently ignored. It has a broad depressed form like a trilobite. It has simple antennules, biramous antennæ, and mandibles like a trilobite, and the gnathobases of the last two function as mouth-organs. An hypostoma is present, and there is a growing point, as is evidenced by the way new segments are added. It is not, it is true, a trilobite, but it looks like a trilobite modified by suppression, and taken in connection with other evidence, certainly does no injury to the theory that the higher Crustacea were derived from the trilobites.

The Malacostraca can be mentioned but briefly. Their most ancient representative whose appendages are known, *Hymenocaris* from the Middle Cambrian of British Columbia, had biramous appendages like those of the trilobites, and most of the modern members of the group have similar ones on some part of the body. As in lower crustaceans, when the exopodites are lost or degenerate, epipodites are developed to replace them, and thus the limbs become variously modified. It is remarkable, however, how close a resemblance there is between the appendages of a trilobite and those of fresh-water syncarids from Tasmania, and even in the Decapoda the seven segments of the walking leg are serially homologous with the seven segments of that of the trilobite. It

has frequently been objected to the trilobites as ancestors of the Crustacea, that they had wide pleural extensions and a large pygidium. To the first it may now be replied that some trilobites did get rid of the pleural extensions, but that, on the other hand, most Crustacea retain some remnants of them. I have already shown above that the large pygidium in trilobites was more primitive than the trunk with numerous free segments, and it may further be pointed out that some orders of Isopoda do have a pygidium.

### ARACHNIDA

My task in this class is rendered somewhat easier by the fact that the followers of Lankester appear to have accepted his explanation of the descent of the class from the trilobites. While I agree with the general thesis, I must point out that a certain amount of caution must be used, for the connecting links are not nearly so satisfactory as one would like them to be, and the trilobites are not nearly so closely related even to the Merostomata, as they are to the higher Crustacea.

In the first place, while the Trilobita were probably the ancestors of the Arachnida, they do not themselves belong to that class. Lankester advanced six reasons for placing them in the Arachnida, but the first is the only one having any considerable weight, and is the only one which will be discussed here. This point was that they had only one pair, apart from the eyes, of pre-oral appendages, while the Crustacea have two pairs. Researches since Lankester's article was written seem to show that this apparent difference between the Arachnida and Crustacea is not fundamental, for the cheliceræ of the former are probably to be homologized with the antennæ, not the antennules of the latter, so that the mouth is in the same position in relation to the appendages in both groups. Further, the mouth does not occupy a constant position in the trilobites, but with the elongation of the hypostoma, is pushed backward, so that from one to four pairs of appendages may be attached in front of it.

If dorsal tests only be considered, one can pick out an excellent series showing gradations from a trilobite into *Limulus*. Thus, there are in the Middle and Upper Cambrian the Aglaspidæ, with *Limulus*-like head, trilobite-like free thoracic segments, and *Limulus*-like telson. Following the history of the group through the Paleozoic, there is, in the Silurian, *Neolimulus* with a head which is surely that of *Limulus* but which has vestigial facial sutures, and free thoracic segments are present. By Devonian times, the thorax had begun to fuse into a shield, although some of the Pennsylvanian species retained a few free segments at the anterior end of the thorax. This series is so convincing that one must believe that the Xiphosura developed from the trilobites, but a study of the appendages shows that there are greater differences between those of a trilobite and *Limulus* than between those of a trilobite and one of the highest Crustacea.

The greatest difference is in the complete lack, at any stage of development, of the antennules, from which it follows that the anterior shield in the Xiphosura is a cephalothorax in which at least seven segments are incorporated. It is, of course, entirely possible that in one line of evolution of the trilobites the antennules were lost and the antennæ developed as chelicerae, but if the test be applied to the Aglaspidæ, the results are at variance with the expectation. Walcott has found Aglaspidæ with appendages in the Middle Cambrian, and they seem to have five pairs of appendages on the cephalon, two pairs of which are elongate, multi-segmented, tactile antennæ, and biramous appendages are present on the thorax.[4] These animals were still Crustacea, and the development of elongate antennæ instead of cheliceræ shows that they were not tending in the direction of the Xiphosura. It is possible, however, that when the appendages of more Aglaspidæ are known, it will be found that some of them showed a tendency to lose the antennules and develop cheliceræ.

[4] Smithsonian Miscl. Coll., Vol. 57, No. 6, 1912.

Some progress has been made toward the connection of the trilobites with the Merostomata, as the Limulava of Walcott are, in a certain sense, intermediate between the two. The Limulava are, however, true crustaceans, for they have five pairs of cephalic appendages, the first of which are elongate uniramous antennules, and also biramous, trilobite-like limbs on the anterior part of the thorax. They are especially trilobite-like in the fact that the antennæ are not elongate tactile organs, but, in *Emeraldella* at least, are biramous. The relationship to the Merostomata is expressed in the shape of the head, body and telson, and the grouping of the cephalic appendages about the mouth. If these animals lost the antennules, developed the antennæ into cheliceræ, added two thoracic segments to the cephalon, modified the appendages, and added a sternal operculum, a merostome would be produced. I think it is obvious that to change a trilobite into a marine arachnid is a more complicated process than to change one into a crustacean of any kind.

To compare the spiders directly with the trilobites may seem somewhat fanciful, yet in some respects the spiders are more trilobite-like than *Limulus* is. On the germ band there is a pair of buds in front of the mouth which probably are antennules. These later fuse to form the rostrum, and the cheliceræ move into a pre-oral position. Moreover, Jaworowski has shown that the pedipalps on the germ band of *Trochosa singoriensis* are biramous. In young spiders the abdomen is segmented, and the anterior segments bear pairs of limb-buds, some of which are later lost, while others develop into lung-books or spinnerets. The number of abdominal segments appears to be variable, from eight to fourteen, another feature which suggests the trilobites. The spiders very probably did not spring directly from this group, but will eventually be traced to it, and not through the Xiphosura.

### Insecta

I quite agree with Crampton that Handlirsch has presented little or no evidence that the Insecta were derived directly from the trilobites. His chief point was that the most ancient known insects, the Palæodictyoptera, were amphibious, and that their larvae, which lived in water, were very like the adult. His second was that the wings of the Palæodictyoptera probably worked up and down only, and that the two main wings were homlogous with rudimentary winglike outgrowths on each segment of the body. These outgrowths resemble the pleural lobes of trilobites, and were considered to have been derived from them. Comstock, who has recently reviewed the question, does not see any evidence that the Palæodictyoptera were amphibious, and I do not think any entomologist or paleontologist has accepted the idea of a direct transformation of pleural extensions of segments of trilobites into wings. The "para-notal" theory certainly does not involve any such conception. That the insects are derived indirectly from the trilobites is, however, entirely possible, and Professor Crampton has marshalled the data for one such possible line of derivation through the Crustacea in the article to which allusion was made in the opening sentences of this essay. Another theory is that advanced by Tothill, who suggested that the Insecta arose through some chilopod-like tracheate, rather than directly from a marine organism. Tothill[5] has pointed out that in the germ band, spiracles appear as early as the limb-buds, and may thus indicate a tracheate ancestor for the insects. This is, of course, discounting the possible effect of acceleration on the embryo, but the whole anatomy of the insects indicates long separation from the marine ancestor. The germ band of the chilopod is somewhat more primitive than that of the insect, for in some species both antennules and antennæ are present, and the maxillæ and first maxillipeds are biramous. The

[5] *Am. Jour. Sci.*, Vol. 42, 1916, p. 373.

presence of two pairs of antennae does not point directly to the trilobites, but to some offshoot like *Marrella,* and it is possible that the line has been: trilobite, *Marrella*-like marine animal, chilopod-like tracheate, insect.

There remain only the Diplopoda, which show a few trilobite-like characteristics, notably their lateral outgrowths and the endopodite-like walking legs on every segment. Antennules are present, antennæ absent, mandibles and maxillulæ much modified, the latter possibly biramous, and maxillæ absent. The most characteristic feature, the possession of two pairs of limbs on each segment on a part of the trunk, can be shown to have arisen comparatively recently (geologically), for Silurian and Devonian fossils which are undoubted diplopods have a test like that of a trilobite and eyes much like those of a *Phacops.* While there are no close connections, there is nothing to show that the Diplopoda could not have been derived from the Trilobita.

## SUMMARY

After the above survey, it should appear that the trilobites, particularly in respect to their appendages, are more primitive than any other Arthropoda. The chief modifications in other groups are in the nature of reductions, in the loss of whole appendages, of branches or of segments. Extra segments are sometimes added in certain appendages, and new outgrowths, epipodites, are common among the Crustacea. The trilobites have what seems, at first sight, a peculiar and specialized dorsal test, but now that it has been shown that the pleural lobes may be lost and the pygidium reduced to a single segment, and, chiefly, that the wormlike form is not primitive but secondary, they may be viewed in an entirely new light.

In the oldest fossiliferous rocks, Lower Cambrian, trilobites are plentiful, branchiopods rare, and no other arthropods present. A greater differentiation is seen in the Middle Cambrian fauna, due, however, entirely to the

remarkable assemblage found by Walcott at a single locality in British Columbia. In this fauna the Crustacea are represented by trilobites, anostracan and notostracan branchiopods, *Marrella,* Limulava (possible ancestors of the merostomes), Aglaspidæ (possible ancestors of the Xiphosura), and Leptostraca, the most primitive Malacostraca. The Upper Cambrian brought the first true Merostomata. In the Ordovician, Ostracoda and Cirripedia first appear, and in the Silurian the first undoubted Xiphosura, primitive Diplopoda, and Scorpiones. Insecta and air-breathing Arachnoidea, including Araneæ, appear suddenly in the Pennsylvanian (Upper Carboniferous), and the oldest known Chilopoda are found with them. All of the tracheates probably have a long pre-Pennsylvanian history, however, and the record of the fossils is liable to be amplified by new discoveries at any time.

The geological record, so far as it is now available, is in favor of the theory that the other Arthropoda were derived from the trilobites, for although Crustacea were highly diversified by the Middle Cambrian, all other than these were rare, and the trilobites, while they had not reached their highest development, were exceedingly abundant and varied.

If the trilobites were the most primitive arthropods, the question of the ancestry of the phylum resolves itself into a search for the progenitors of the former. What would be the form of the animal from which the trilobite was derived? The depressed form universal in the subclass and the equally universal lateral ("pleural") lobes have already been commented upon. From a study of comparative morphology, it appears that the more ancient trilobites, and the more ancient members of higher families within the subclass, have the most nearly flat form, the narrowest axial, and the widest pleural lobes. Turning to ontogeny, it is found that in most cases the protaspis of any species shows the same characteristics. All these suggest a broad depressed animal with narrow

axial portion as the ancestor. Only a few specialized trilobites like the Remopleuridæ and one subfamily of the Cheiruridæ show extensive reduction of the pleural lobes.

A study of the ontogeny of trilobites with both large and small pygidial shields shows that the pygidia are proportionally larger in the protaspis than in the adult, and the more ancient forms pass through a stage in which they are practically isopygous. This suggests that the ancestor had subequal cephalic and abdominal shields. Since the thorax grows by the breaking down of the pygidium, the ancestor should lack free thoracic segments. Curiously enough, among Walcott's remarkable finds in the Middle Cambrian there is an isopygous crustacean without free thoracic segments. It was named *Naraoia* and referred to the Branchiopoda by Walcott,[6] but since it satisfies the theoretical considerations and evidently can not be referred properly to any other subclass, I am inclined to look upon it as the simplest of all trilobites. The specimens so far described are not fully preserved, but the appendages are apparently biramous and trilobite-like, and there are at least three pairs on the head and fourteen on the pygidium. From Agnostidae with subequal shields and two thoracic segments to *Naraoia* with subequal shields and no thoracic segments is but a step. If the pygidium were built up by the coalescence of once free segments, that step would be in the direction of specialization, but since the reverse is the case, the extraordinary conclusion is reached that the simplest trilobite did not look at all like the traditional benthonic round annelid.

The study of the ontogeny of many species of trilobites long ago established the fact that in the ontogeny the eyes, which may be entirely absent in the very young of the simplest oculiferous species, appear first on the anterior margin and during growth move backward on the head. A point which has not been noted is that this movement is correlated with a backward movement of

[6] Smithsonian Miscl. Coll., Vol. 57, No. 6, 1912, p. 175.

the mouth, as indicated by the increase in length of the hypostoma, and an increase in size of the anterior part of the glabella. There is nothing mysterious about the process, as it is probably due to the increase in the size of the anterior (digestive) portion of the "stomach." This indicates that in the ancestral form the mouth and eyes were close to the anterior margin, and does away with the necessity of the bent annelid to explain their migration. Many of the simpler trilobites are of course blind, as is *Naraoia*.

It is true that much of the argument involves the use of principles drawn from the study of ontogeny, but where the ontogeny points to animals which actually exist and helps to explain observed facts, its use seems to be justified. Swinnerton has recently suggested that students of the ontogeny of trilobites have been led into an entirely wrong interpretation because they have not realized that the protaspis, like the nauplius, is a specialized larva adapted to a nektonic mode of life. Since Swinnerton believes that the trilobites are descended from benthonic annelids, one can not but wonder why the nektonic trochophore was not carried over instead of requiring the development of a new and totally different free-swimming larva by the trilobites. All indications derived from the present study are that the primitive trilobites were floating and swimming animals, that their adoption of a crawling habit was a specialization, that the protaspis was nektonic because the adults were, and that the nauplius of recent Crustacea is a similar free-swimming larva because it harks back to ancestral conditions.

# THE UTILIZATION OF ECHINODERMS AND OF GASTEROPOD MOLLUSKS

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The Puget Sound region, in the State of Washington, is noted for the wonderful abundance and diversity of its fauna. The region is also noted for its several groups of archipelagoes, of which the San Juan Archipelago is an especially beautiful one.

Around the shores of these islands, echinoderms are found in great profusion. Particularly noticeable are the common forms of starfish, sea urchins, and sea cucumbers. The most common starfish are *Piaster ochraceus* and *Evasterias troschelli,* which show, respectively, considerable substantive and merestic variation. In the environs of Bremerton, the latter finds more congenial conditions than any of the other common species, and there it occurs in a ratio of 25 to 4 of the former, while in the San Juan island group, *P. ochraceus* is by far the most numerous. Besides these two species, *P. paucispinus* and many others are also found, but in smaller numbers. The twenty-rayed starfish, *Pycnopodia helianthoides,* occurs quite plentifully at various places, *e.g.,* Bremerton, Griffin Bay, East Sound, etc. Sea urchins, *Strongylocentrotus dröbachiensis, S. purpuratus, S. franciscanus* are very numerous, especially the former. At low-water, *S. dröbachiensis* may be seen in the bays of the northern part of the sound in large patches, and at a depth of only four meters. *S. franciscanus,* which becomes very large—7 to 13 centimeters in diameter—is found just below low-water mark; I have seen it in large numbers in the vicinity of the Biological Station at Friday Harbor. The most noticeable species of sea cucumbers are *Cucumaria japonica* (Semper), *C. chrondjelmi* (Theil), and *Stichopus cali-*

*fornicus* (Stimpson) Edwards. *C. chrondjelmi* is exceedingly abundant near the Sucia Islands. All these species may be obtained by dredging, and *C. japonica* may be picked by hand at low-tide.

Of all the echinoderms, common starfish, *Piaster, Evasterias,* etc., are most easily obtained. They occur within the lower limit of the average ebb-tide, and sometimes in such profusion that, especially when the stars are brightly colored, they may be seen at half a mile's distance. Their occurrence is independent of town sites, being determined by the nature of food available. Shores well supplied with barnacles usually have a large number of starfish. And the fact that they are abundant at a distance from towns adds to the desirability of their use as a food product. The parts of the starfish and sea urchins utilizable as food are the gonads. During the breeding season, these grow enormously, so that in the starfish the body becomes twice its normal size, the gonads completely filling the gastric cavity. The part of the sea cucumber utilizable as food is the muscles.

Echinoderm gonads as a food commodity would be the object of an industry of annual periodicity like the salmon industry. As the spawning season of starfish and sea urchins comes in the spring, the canning of the roe could be well completed before the salmon season begins; or the making of echinoderm gonads into caviar might well be done along with the canning of fish, whether salmon or otherwise. The gonads of the various species of the larger starfish are ripe in April; those of the sea urchin, in June, as regards species of the north Pacific coast (Figs. 1–3).

There can be no question about the advisability of using the spawn and muscles of echinoderm as food, even in a country where all kinds of food are as plentiful as in the United States. The question is rather how to utilize this part of nature's storehouse to the best advantage for mankind.

Barbier (1908) states that the natives of Madagascar

have developed a considerable industry in the utilization of starfish, sea urchins, and sea cucumbers as food. In 1902, the marketable quantity of sea cucumbers represented a value of 175,000 francs. The province of Tulear produced alone 30 tons, but the lack of necessary labor prevented further production that year.

Taylor (1908) reports an interesting fact, namely, that

FIG. 1. Mature ovary of *Evasterias troschelii*, ½ natural size. (Photo by author.)

the Arctic Fox of the Aleutian Islands, so highly valued for its beautiful fur, feeds, in winter, on echinoderms, *e.g.*, sea urchins.

Reagan (1907) claims that the sea urchin (*Strongylocentrotus dröbachiensis*) is used by the Pacific coast Indians as food.

In conversation with the United States Commissioner of Fisheries (1916) I learned that the roe of starfish is being used in France as food and also as bait in the sardine fisheries; and through Professor Kincaid, I am informed that certain species of sea urchins, which in the market of Naples are called "Frutta di Mare," and in the West Indies "Sea Eggs," are sold as food, but I have not

had the opportunity to consult literature on these points.

Brunchorst (1898) shows that a number of excellent food fish common to the coast of Norway feed on echinoderms, and mentions especially: *Anarrhichas lupus,* and *A. minor; Lycodes esmarckii; Pleuronectes microcephalus,* and *P. platessa;* others not used as food fish but which feed on echinoderms are, *A. latifrons, P. cynoglossus,* and *Galeus vulgarus.* Perhaps other food fish such as *Gadus callarias* and *G. pollachius* also feed on echinoderms, as I have at least found at times small starfish in their stomachs.

Carr (1907) found that out of 150 starry rays (*Raia radiata*) ten contained echinoderms (*Asterias, Echinus,*

Fig. 2. Semi-mature ovary (largest) and spermary (smallest) of *Piaster paucispinus,* 2/3 natural size. (Photo by author.)

and *Ophiocoma*), and out of 12 haddock (*Gadus æglefinus*) two contained echinoderms (*Ophiocoma*). Three out of 13 wolffish (*Anarrhichas lupus*) contained echinoderms. In 1908, the same author tabulated observations on 370 common dab (*Pleuronectes limanda*) and showed that out of this number, 56 contained echinoderms, *e.g.,* Ophiuroids and Echinoids; 5 long rough dab (*Hippoglossus liman-*

*dioides*) out of 60 fish contained Ophiuroids; 10 out of 25 *G. æglefinus,* contained echinoderms (Ophiuroids and Echinocyamus), and one gray gurnard (*Trigla gurnardes*) out of 150 fish also contained echinoderms.

From the findings of Brunchorst and Carr it is seen that various kinds of fish feed on echinoderms, whence the suggestion that echinoderms be used as bait. How-

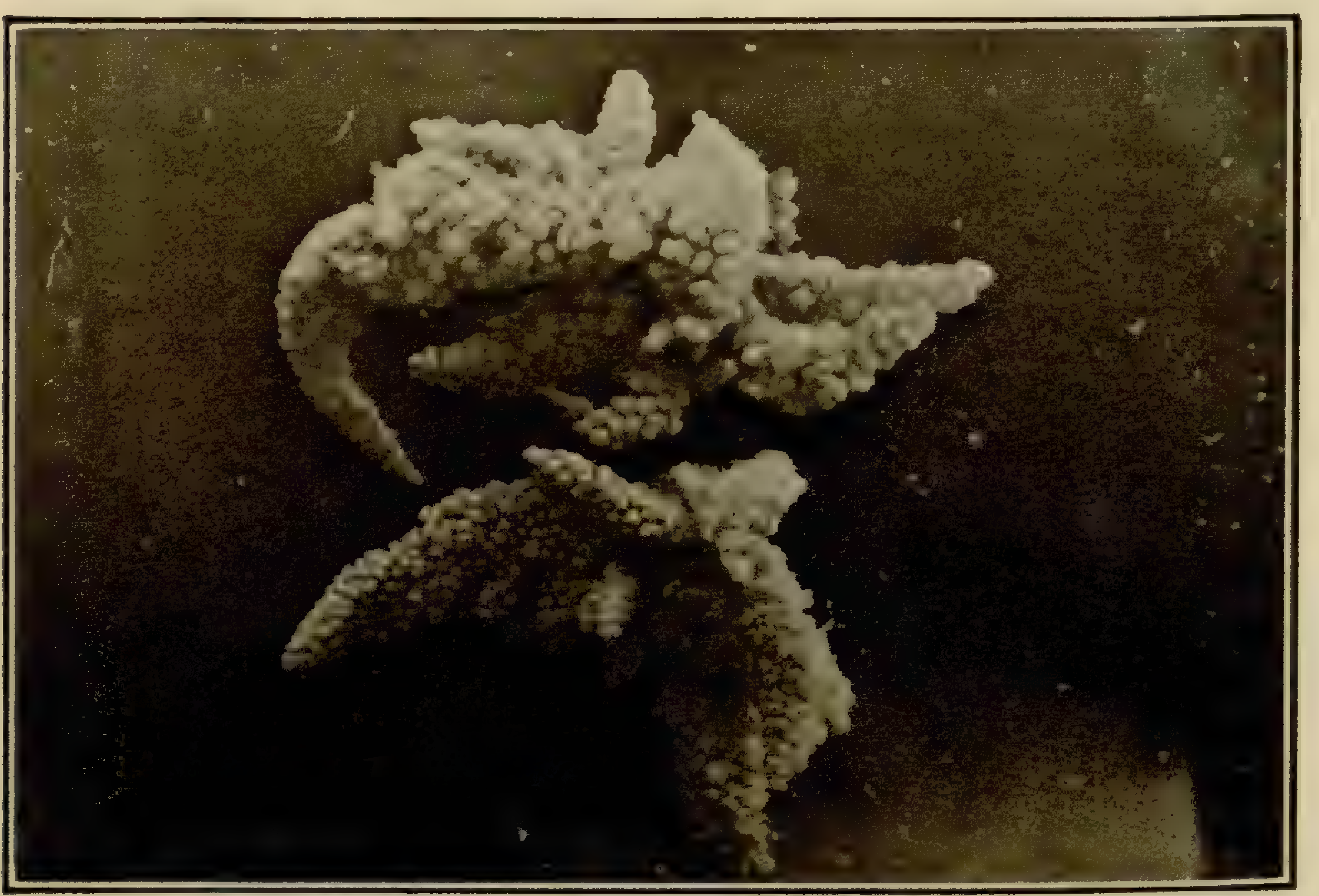

FIG. 3. Mature ovary and spermary of medium-sized twenty-rayed starfish, *Pycnopodia helianthoides,* $\frac{2}{3}$ natural size. (Photo by author.) N.B. The alveoli are larger in the ovary.

ever, it may be that for a number of forms echinoderms are resorted to only when other food is out of reach, though the common dab, according to Carr, appears to eat them during the greater part of the year. The long rough dab feeds less on echinoderms than the common dab, perhaps owing to a difference in migration habits of the two.

A large part of an echinoderm industry would be biproducts, since the main bulk of the starfish consists of material best suited for guano. No absolute waste material need remain; all of the animal may be utilized. Indirectly, the shell-fish industries would be benefited by

reducing the number of starfish in regions where shell-fish live, as various forms of starfish feed on marketable shell-fish. Lebour (1916) makes an interesting statement:

> The mussels on this coast have not many enemies, but by far the most important of these is the starfish, *Asterias rubens,* which constantly preys upon them. A formerly flourishing bed near the Tyne has lately been exterminated by this starfish and it is a bad enemy everywhere. *Purpura lapillus* [a small gasteropod] devours the mussels on the scaup, Holy Island, and here in parts the devastation caused by this small and very destructive mollusk is great. It does not, however, appear to be a scourge elsewhere. The only possible way of dealing with such foes would be to destroy all starfish whenever found, and to collect the *Purpura lapillus* systematically, and also its spawn, and destroy both.

Kellogg (1910) records nothing favorable about the starfish. To him it is a pest.

> The removal of these pests has always been a very difficult matter, and no entirely satisfactory method has been devised for accomplishing it.

When the *economic value* of starfish is realized, depletion of starfish may result from overfishing, and "to destroy all starfish whenever found" will be out of the question. If it prove necessary to protect starfish, where only the gonads are to be used, these may be removed on the grounds, while fishing, and the starfish at once put back into the water below low-tide level to avoid unnecessary exposure. The operation of removing the gonads could be carried out successfully without killing the animal, since echinoderms possess great regenerative powers. As male gonads may be unfit for caviar, it is worth noting that they can be easily distinguished from the female since the latter are of pinkish color while the former are of light yellowish hue. The reproductive power, and growth of starfish, are very great. According to Kellogg, "A female starfish may, if large enough [depending on amount of food] begin to extrude eggs during its second summer, and many by that time attain the required size."

As echinoderms, on the north Pacific coast, can be more easily caught than any other kind of sea food, the starfish and some of the sea cucumbers may simply be picked

up at low-tide, and sea urchins and certain sea cucumbers may be obtained by dredging, the expenses connected with their utilization as a whole should be comparatively low,

Fig. 4. Live shells of *Polynices lewisii*, natural size. (Photo by author.)

making it possible to sell the products at a reasonable price.

In quoting Lebour, I mentioned *Purpura lapillus* as a destructive enemy of bivalve mollusks. I now wish to point out the possibility of utilizing destructive gasteropods:

*Polynices lewisii*, a very large gasterpod (Fig. 4), is a great destroyer of mollusks of commercial value. Its foot may reach the length of 21 centimeters and a width of 13 centimeters, and a depth of the body about 10½ centimeters. It destroys oyster beds by its burrowing in them in search of clams, but it is not known whether it attacks oysters directly. On account of its burrowing habits, the oystermen, at the head waters of Puget Sound, destroy large numbers of them.

Keep (1888), speaking of *Lunatia lewisii* Gld., now merged in *Polynices*, claims that it possesses a flint drill

which it carries in its mouth, and by use of which it drills into the clam or whatever mollusk it may encounter, killing the same. This, it is claimed, is a common habit of members of the family Naticidæ of which the genera *Natica* and *Lunatica* are best known. Daugherty (1912) says:

> *Natica* is another drilling sea-snail common to our coast. It burrows in the sand for clams and bores a hole with its radula, rotating its own body in the action.

Agersborg (1918), during the summer of 1916, observed a number of specimens of *Polynices* in the actual act of killing and eating clams. At low-tide, when rowing along the shores of Dyes Inlet near Chico, Washington, a large number of *Polynices* was found. As the tide was very low it was possible to pick them up by using a dip-net. Some of them, however, were not so easily removed from the bottom as others, holding to the same by means of the enormous foot, or having sucked down into the sand to the depth of about ten centimeters, leaving only part of the shell uncovered in the middle of a pit. It was soon found that there was a definite cause for their holding on to the bottom so firmly; these individuals of *Polynices* were feeding. The process of feeding was found to be somewhat different from that described by Keep, and Daugherty.

As *Polynices* crawls along the bottom it kills any clam it encounters by suffocation. The soft-shelled clam, *Mya arenaria*, which is quite numerous in the bays of Puget Sound, is a common victim. Hard-shelled clams, *Paphia staminea, Cardium corbis*, are also an easy prey for this ravener. In the case of *Mya*, the gasteropod sucks itself over the syphon down into the sand until its victim is dead from suffocation, and then when the clam has opened, *Polynices* simply sends its proboscis between the valves and devours the content. As for the hard-shelled clams, the process of feeding is similar to that used when eating a *Mya* but the method of killing is different. In this case the prey is held in the "sole" of the foot until the

adductor muscles are relaxed or the victim is dead, when the feeding begins. Several dead clams, of these species mentioned, were found in possession of *Polynices,* but none of them were drilled. It is thus seen that this gasteropod is very decidedly an enemy of the bivalved mollusks, but its method of killing clams is different from that described by Keep and Daugherty.

Several specimens of *Polynices lewisii* were obtained and brought to Bremerton, where experimentation on the possibility of utilizing them as food was carried out. Two methods of preparing the animals for the table were used: first, steaming in the moisture contained within its swollen foot, and second, breaking the shell and frying the animal alive in butter. Either of these methods gave good results. By the former, a delicious broth was the principal result; by the latter, a large piece of variant meat. The foot, however, by either method, becomes rather tough when cooked. As some one has held that the meat of *Polynices* is poisonous, not so very much was eaten; no ill effects, however, were felt from that consumed. The idea that *Polynices* is unfit for food is of course baseless, as I am informed by Professor Kincaid that thousands of *Polynices* shells may be found in the Indian kitchen-middies, which indicates that the Indians used this mollusk as food, and as the *Polynices'* shells are found in these remains in much greater proportion than any other shells, this gasteropod must have been widely sought by the Aborigines; or it may be that *Polynices* was formerly more abundant than any other mollusk on our western coast. At any rate, this gasteropod seems to have been a common diet of the Indians who lived along Puget Sound. The tastes of Indian and white man are not unlike in these matters, for white people eat various species of clams, also an Indian diet, and seem to delight in such food, as is well demonstrated by the establishment of shell-fish canneries on our coasts.

It does not seem unreasonable, therefore, that *Polynices* as well will find a ready market. In fact, it might

well be prepared as an extra delicacy and sold as such, and in that way made to make up partly for injuries that it inflicts on the bivalve-mollusks.

Barbier (1908) enumerates a large number of gasteropod mollusks used by the natives of Madagascar in various ways. Not only is the animal matter used as food, but the shells are commercialized as well. Having enumerated ten species of the genus *Murex*, and 139 species from different genera including *Littorina, Nerita, Cypræa, Pterocera, Strombus, Neritina, Turbo, Conus, Terebra, Natica, Cassis, Harpa, Mitra, Voluta, Vasum, Oliva, Fasciolaria, Purpura, Rapana, Eburna, Nassa, Ranella, Triton, Fusus, Neptuna, Busycon,* and *Pyrula*, all of which are marine forms, he adds the following terrestrial and freshwater gasteropods: *Helix hæmastoma* L., *Bulimus perversus* L., *Mulimulus multilineatus* Say, *Pupa uva* L., *Clausilia cana* Gld., *Auriculus auris* Midæ L., *Tudora versicolor* Pfr., and *Helicina miltochila* Cross, and says:

Tous ces coquillages servent à la nourriture des indigènes qui mangent leur chair cuite dans la coquille sur un feu ardent sans acun assaisonnement.

It is worth noting that genus *Purpura* which causes great destruction of the mussel beds on the English coast, and which was suggested by Lebour to be systematically collected and destroyed, serves the Indians of Madagascar as food. *Murex, Natica, Nassa, Busycon,* and others related to the gasteropod types on our coasts are being utilized as food by the natives of Madagascar. *Cycotypus canaliculatus* Verrill & Smith, 1873 (*Busycon canaliculatus* Say, or *Fulgur canaliculata* Gould, 1887, Dall, 1889), is a very pernicious enemy of oysters. According to Sumner, Osburn and Cole, 1908,

It is abundant in shallower water generally . . . pretty generally distributed throughout Buzzard Bay and Vineyard Sound. It preys upon mollusks and is said to be destructive to oysters (p. 707).

It is still of little commercial value save that of being used for dissecting purposes, and some Europeans, in New

England, have ventured to use it as food, but this is by no means a common practise. The genus *Urosalpinx* is closely allied to *Murex*; several of its species are found on the east coast of the United States. Arnold (1916) says of *Urosalpinx cinerea:*

> This well-known species is regarded by Chesapeake and Long Island Sound oystermen much in the light of a plague. These active predaceous mollusks live upon bivalves, and preferably upon oysters. They bore a small hole through the shell of their helpless victims, and then proceed to extract the succulent, fleshy animal from within. The oystermen call them by the suggestive name of "drill," and wage incessant warfare upon them.

Daugherty claims: "It is a feeder upon oysters;" and Kellogg says in part:

> There are several species of the snails that are destructive to bivalves. Among these the large winkles or conchs of northern shores do very little damage; but some of the smaller forms, particularly the oyster drill, cause large losses here and there along the Atlantic coast. . . . The drill, or *Urosalpinx,* is most destructive to young oysters. It seems to be unable to bore through the shell of large individuals. . . . Like starfish, oyster drills were formerly not numerous on the New England oyster beds, but in recent years have increased greatly. In New York Bay, and in the Chesapeake, they are abundant . . . in Louisiana, a larger drill, *Purpura floridana,* is sometimes very destructive.

Opinions thus seem to vary as to the destructive habits of some of the gasteropods upon bivalve mollusks, but the findings of Dr. Copeland (1916) are very conclusive: *Busycon* reacts positively toward oyster juice, in fact, the oyster often forms a conspicuous part of its natural diet. All the investigators, however, seem to agree about the habits of *Urosalpinx.* In view of the fact that these gasteropods *are* esculent, injurious to other marketable mollusks, near large cities, and generally easily obtained, it seems rather strange that they are seldom found in the market. *Polynices lewisii,* which is still quite abundant in the upper part of Puget Sound, is generally destroyed by the oystermen whenever found in the vicinity of oyster fields. As a natural enemy, *P. lewisii* seems to have none more dangerous than the twenty-rayed starfish (*Pycno-*

*podia helianthoides*). As a matter of fact, bays that have none or very few *Pycnopodia* may have a large number of *Polynices,* and bays that are well populated with this starfish have remarkably few *Polynices* present. When I later experimented on the sensitivity of *Polynices* to *Pycnopodia* (Agersborg, 1918) I found in all instances, that when the slug came into contact with the star, it withdrew its foot at once. The monstrous foot, though it seemed impossible that it could be withdrawn within the shell, was very quickly covered thereby. Upon withdrawing the foot in a hurry, as it does when in contact with *Pycnopodia,* the periphery of the foot, which is perforated, throws a spray like a garden sprinkler with the holes in the spray-disk plugged except those around the periphery. No matter how much larger the animal is than its shell, when all the water is squeezed out of the foot, the former can be completely covered by the latter. In such a condition, however, *Polynices* can not live very long. It is itself easily exhausted when completely shut up within its shell. If it is not allowed to take in fresh water supply when it comes out to breathe it soon relaxes, an easy prey to the gluttonous *Pycnopodia.* In fact, when leaving *Polynices* with *Pycnopodia* in an aquarium, two of the former were killed and eaten by the latter within three days, leaving the shells and opercula.

The absence of *Polynices* where *Pycnopodia* abounds, together with the facts observed when keeping the two in the same aquarium, seems to indicate definitely that *Pycnopodia* preys on *Polynices.* As mentioned above, *Polynices* is a nuisance to oyster growers, even if it does not feed on oysters, for it destroys the oyster beds by burrowing in them; primarily *Pycnopodia* is a gasteropod feeder, and though it is quite omnivorous and may feed on anything it happens to encounter, it is not known whether it feeds on oysters. The question then is: might not *Pycnopodia* be used as a check against *Polynices* in the oyster beds? This could easily be tested out experimentally: *Pycnopodia* could be placed on oyster beds to

see whether it remains there or crawls away. If the latter was found to be the case then *Pycnopodia* is not adapted to feed on oysters and might then be kept on the outside of the oyster beds as a guard against the inroad of *Polynices*.

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# THE VIBRATILE ORAL MEMBRANES OF GLAUCOMA SCINTILLANS, EHR.

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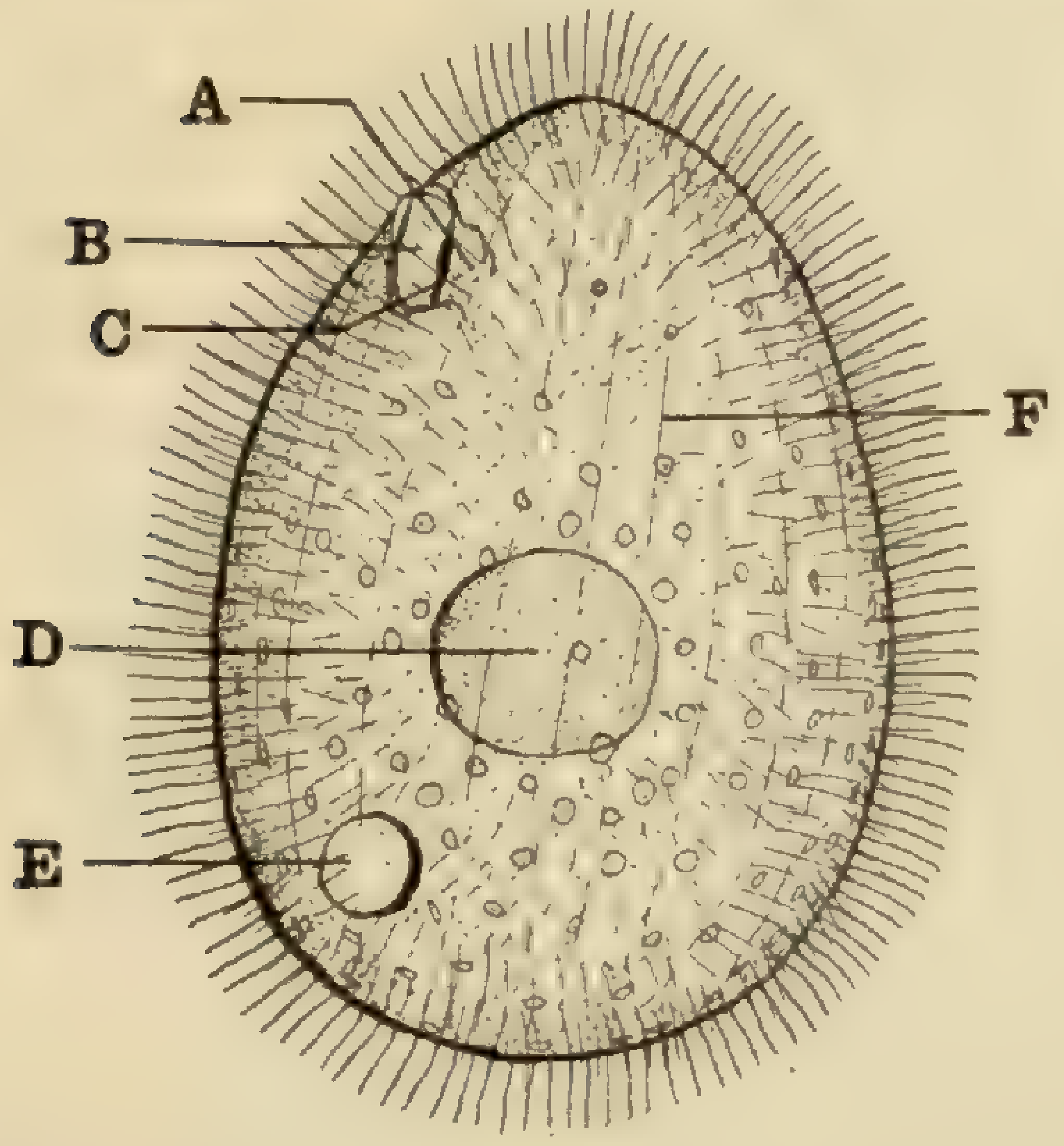
*Glaucoma scintillans* was first described by Ehrenberg in 1838, and has received since that time but little notice, save as a species to be included in catalogues of microfauna. The writer has found it of interest because of the peculiar appendages borne about the mouth, *i.e.,* the vibratile oral membranes. Other species also bear either one or two oral membranes, but *Glaucoma* is distinct in this respect: that the membranes completely encircle the oral aperture; are distinctly bilabial in form, and are usually in active, characteristic motion. So rapid is the motion of the membranes that, due to the reflection and refraction of the light rays from the microscope mirror which is produced, a twinkling or scintillating appearance results. It is from this phenomenon that the creature has received its specific name, *scintillans.*

The body of *Glaucoma* is ovate (Figs. 1 and 2), the ventral surface flattened and entirely ciliated; the dorsal surface arched, and naked. The cuticle is everywhere longitudinally striated. The oral aperture lies in the anterior half of the ventral surface, its longer axis slightly oblique, though in many individuals the longer oral axis is practically parallel with the longer axis of the body. The nucleus is spherical, its diameter being approximately equal to the long axis of the mouth, and situated in the central portion of the body, often, however, slightly nearer the posterior extremity. A secondary nucleus, roughly a fifth or sixth of the diameter of the primary one, can frequently be made out. The contractile vacuole is usually single, rarely double, and situated in the posterior third of the body. In shape, *Glaucoma* is more constant than many of the other cili-

ates. The adults are usually almost perfectly oval or ovate; the young are apt to be often spherical. In size the adults vary between 70 and 75 microns in length. The writer has noted a few individuals that were 78–80

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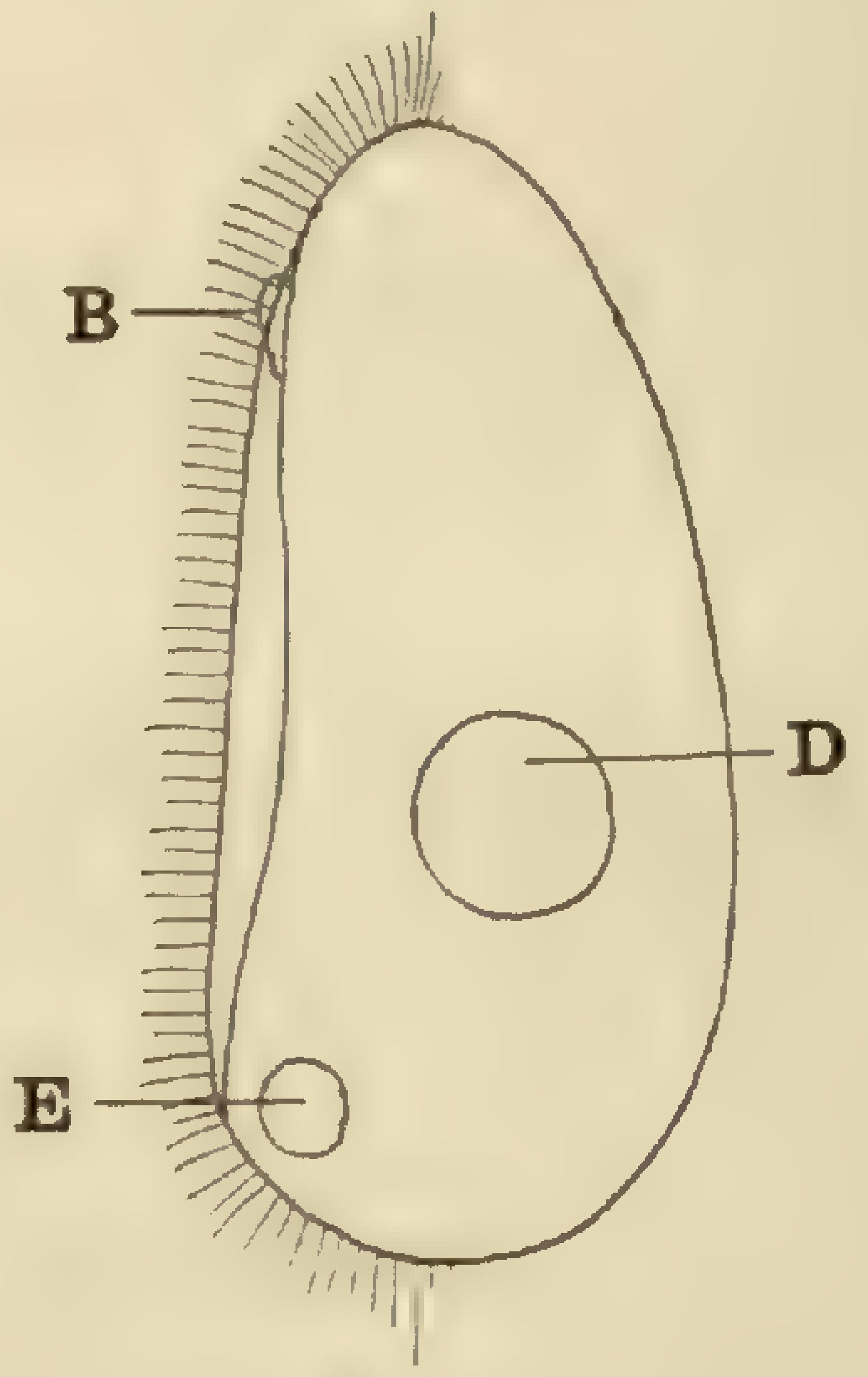


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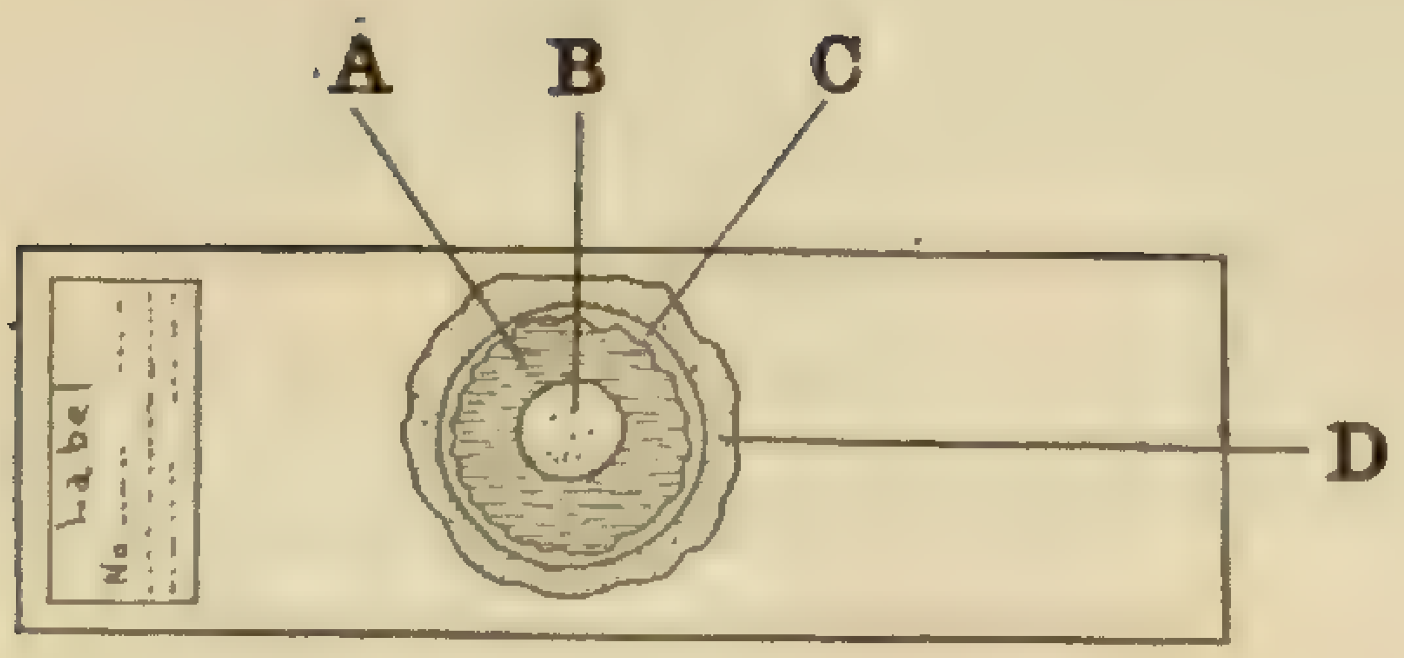


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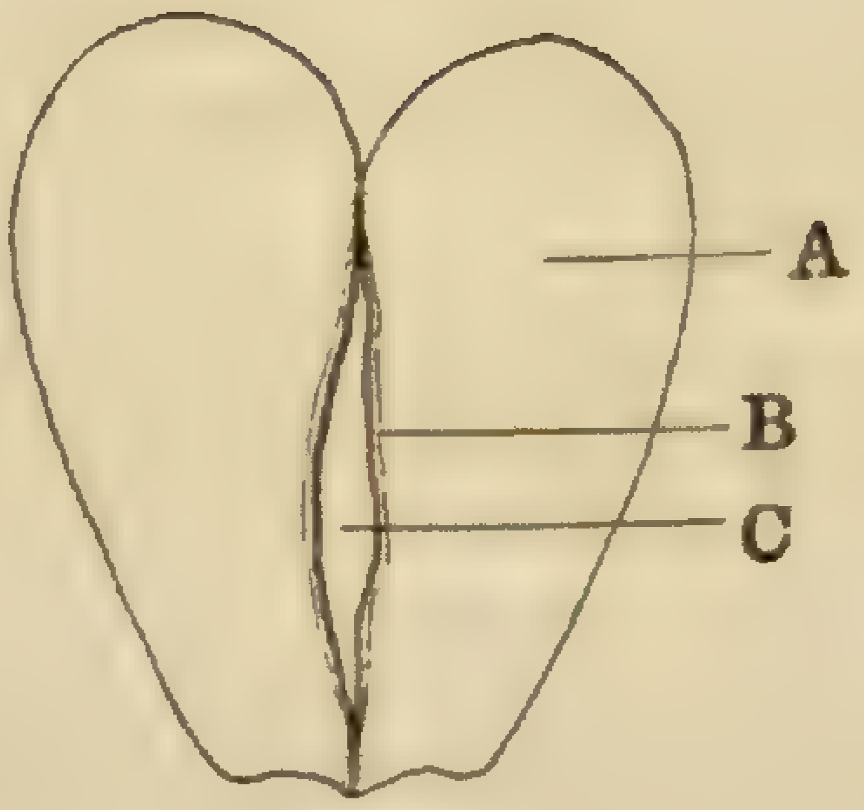


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microns.[1] These, however, were very rare, and did not number more than perhaps a dozen individuals among several hundred.

*Glaucoma* is a fairly common protozoan. Its usual habitat is in stagnant water, particularly pond water in which there is considerable decaying aquatic vegetation. The writer has found it abundant in the brackish water of a peat swamp. It commonly occurs wherever there are also numbers of small flagellates. Samuelson ('57)[2] says that he has reared large numbers of them in an infusion made from cabbage leaves and distilled water. Cultures of *Glaucoma* were prepared in the laboratory by the writer by allowing water, containing cattails, ponds lily leaves, and *Elodea* to become putrid. Numerous *Glaucomæ* were found congregated in the grayish scum that overspread the surface of the infusions, where they were engaged in feeding upon the bacteria, which seems to form the bulk of their food.

It was necessary to keep individual *Glaucomæ* under observation over extended periods of time, and this was accomplished by means of a device whose utility was such as to warrant a description of it here (Fig. 3). A piece of lens paper, or of thin typewriter manifolding paper,

FIG. 1. Mature *Glaucoma scintillans*, ventral aspect. *A*, indefinite gullet; *B*, vibratile oral membranes or outer lips; *C*, opening to buccal cavity (or mouth proper), or inner lips; *D*, nucleus; *E*, contractile vacuole; *F*, striations of the cuticle.

FIG. 2. Outline drawing of the lateral aspect of *Glaucoma scintillans*. *B*, outer lips; *D*, nucleus; *E*, contractile vacuole. Note that the ventral surface may often be depressed.

FIG. 3. Micro-aquarium. *A*, circle of thin lens paper, bearing a circular opening; *B*, *C*, the cover glass: *D*, sealing material (parafin or Canada balsam).

FIG. 4. The vibratile oral membranes, or outer lips, with the opening of the mouth just beneath them. Semi-diagrammatic.

FIG. 5. The outer lips dissociated from their connection with the cuticle. The broken line represents the position of the surface of the cuticle.

FIG. 6. A single outer lip, showing the transverse striations.

FIG. 7. The outer lips represented as being spread out flat, and seen from above. *A*, outer lips; *B*, inner lips; *C*, opening of mouth proper.

[1] Eyferth (1909) gives the length of *Glaucoma scintillans* as lying between the extremes, 20 and 80 microns! The lesser figure probably refers to the length of the young, just after division.

[2] *Quart. Jour. Mic. Sci.*, Vol. 5, 1857, p. 19.

the shape and size of the cover glass, with a small circular opening cut in its center, is soaked in hot parafin, drained, and placed upon a glass slide. After the parafin has cooled a drop of water containing the organisms is placed in the center of the circular opening, and the cover glass applied. The drop should flatten out just sufficiently to fill completely the space between the cover glass and the slide inside the circular opening. The cover glass must now be sealed down about its edge by means of a camel's hair brush dipped in hot paraffine. Such a device the writer has called a *micro-aquarium* and has found its employment of the utmost use. In it scores of *Glaucomæ* were kept alive for as long as eight hours.

For quieting the movements of *Glaucomæ* thick gelatine solution was used, and a drop, mixed with a drop of water containing the organisms, placed on a slide and examined under the 16 mm. objective and allowed to remain uncovered until evaporation had rendered the mixture of such viscosity as to retard the animals sufficiently. The cover glass was then applied and sealed down with either hot paraffine or castor oil to prevent further evaporation.

For killing without staining and without distortion either a one per cent. aqueous solution of copper sulphate, a ten per cent. solution of alcohol, or a fifteen per cent. solution of chloretone was found satisfactory. The best of all killing reagents for *Glaucoma,* however, was found to be a two per cent. aqueous solution of alum.

*Intra-vitam* staining was accomplished with an aqueous solution of Bismarck and methyl green; while for staining after killing there were utilized: iodine, methyl green, methyl blue, Bismarck brown, and safranin.

The appendages which make *Glaucoma* of especial interest are the oral membranes. These are present in the form of two hyaline, lip-like structures, lying one on either side of the buccal cvaity (Fig. 1). For convenience these will be referred to as the lips. In form they are roughly rectangular, and approximately twice as

long as broad, with one extremity enlarged and rounded, and the other slightly narrowed and with a crescentric edge (Fig. 4). They are joined at their base, at either end, and project outward from the body so as to be plainly visible in profile (Fig. 2). Usually the lips are in constant motion, but occasionally may be seen inactive, and when so are normally closed (Fig. 1).

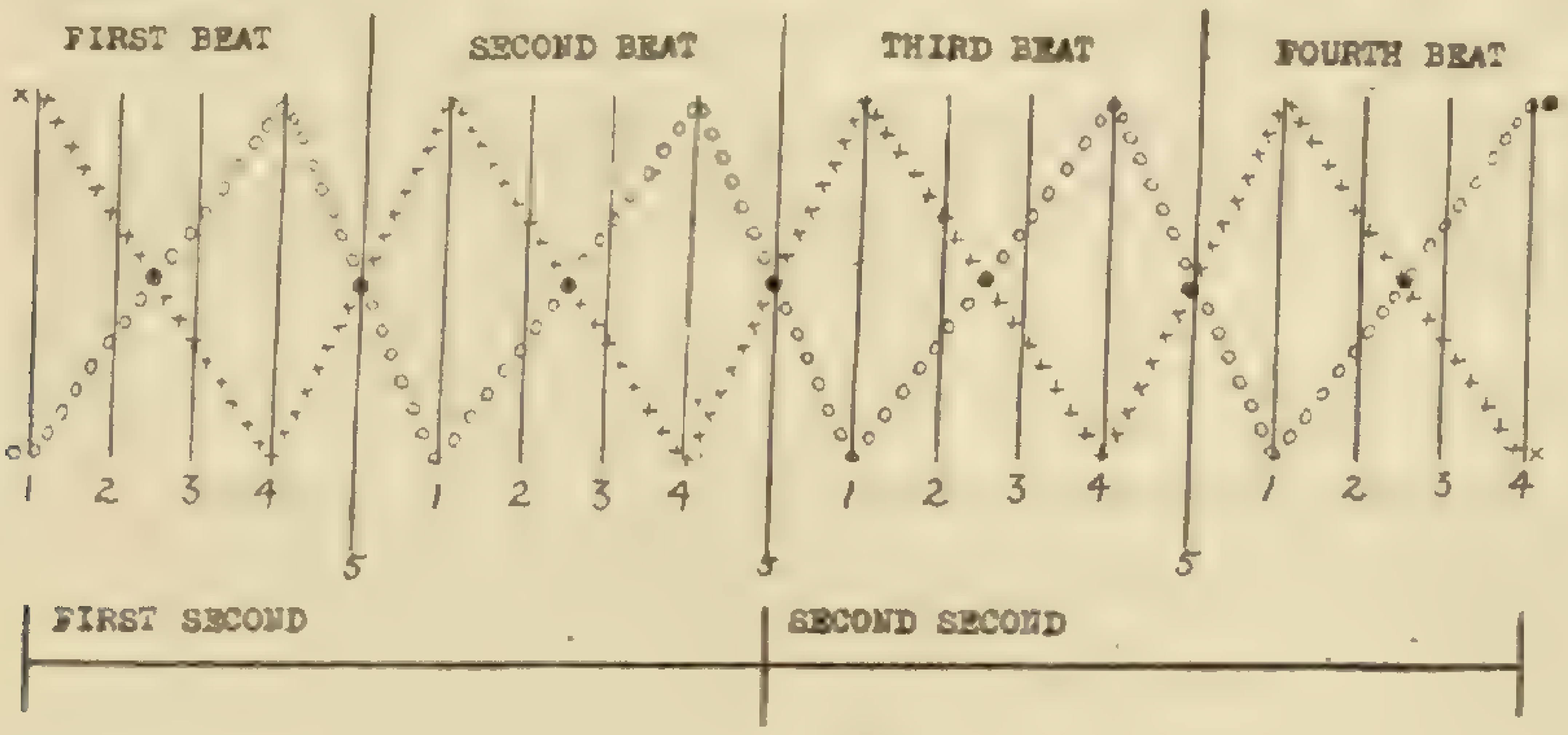
When the body of *Glaucoma* is disintegrated with strong alum solution, or copper sulphate, it is sometimes possible to isolate the two lips together, they apparently being more resistant than the rest of the body. When this is done, it can be seen that they are more nearly oval in outline, possessing an extended, fin-like portion, which projects into, or beneath the cuticle (Figs. 4 and 5).

That the origin of these membranes may be sought for in the fusion of rows of cilia situated on either side of the buccal cavity, is suggested by the fact that each lip exhibits striations running at right angles, or nearly so, to the longitudinal axis (Fig. 6). These striations are broadest at their bases, and narrowest at their tips, and recall to mind the rows of stout oral cilia found among so many of the infusoria.

Between the bases of the lips lies the opening of the buccal cavity, or mouth proper. Its edges, which may be termed the inner lips, are also capable of a motion which will be described later (Fig. 7).

The movement of the outer lips consists of a rapid opening and closing, and is best described by the word, beating. The rate of the beating is apparently under control of the animal, and varies at different times. Under normal conditions the beating of the lips is very rapid. The hyaline membranes, even when at rest, seem to glisten, possibly due to the striations which refract the light in different ways, and when in rapid motion produce the pretty scintillating effect which has given the creature its name. Observations made by the writer of the rate of opening and closing of the lips, showed that it varies from eight or nine times per second, through

all the lessening degrees, to actual cessation. Usually the beating, no matter what the rate, is regular, *i.e.*, the durations of opening and closing being of equal length. This, however, is subject to modification, apparently at will, and individuals are sometimes, though not frequently, encountered in which the lips beat with great

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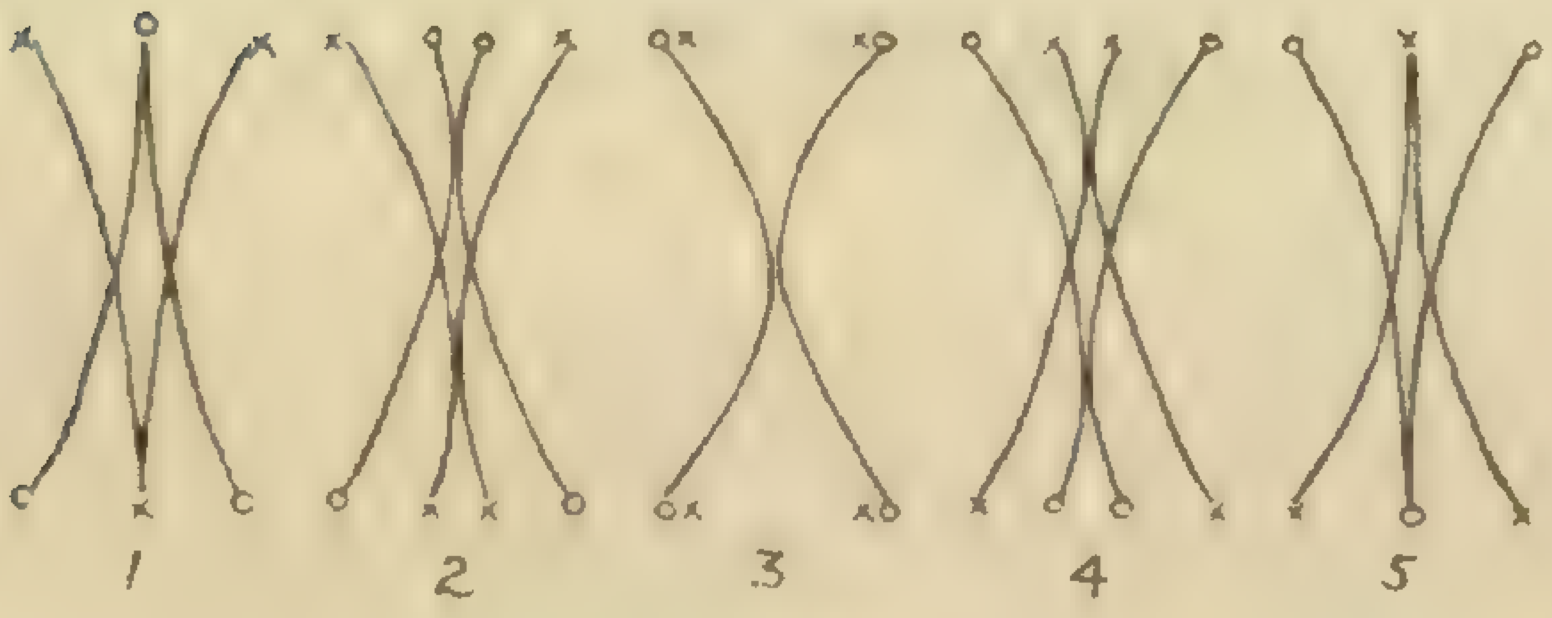

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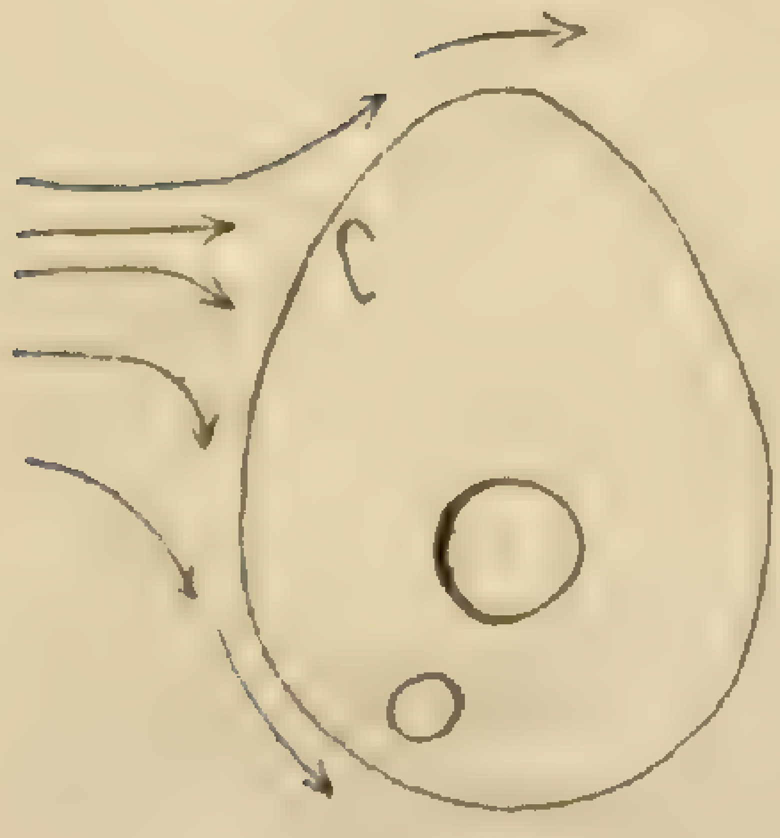

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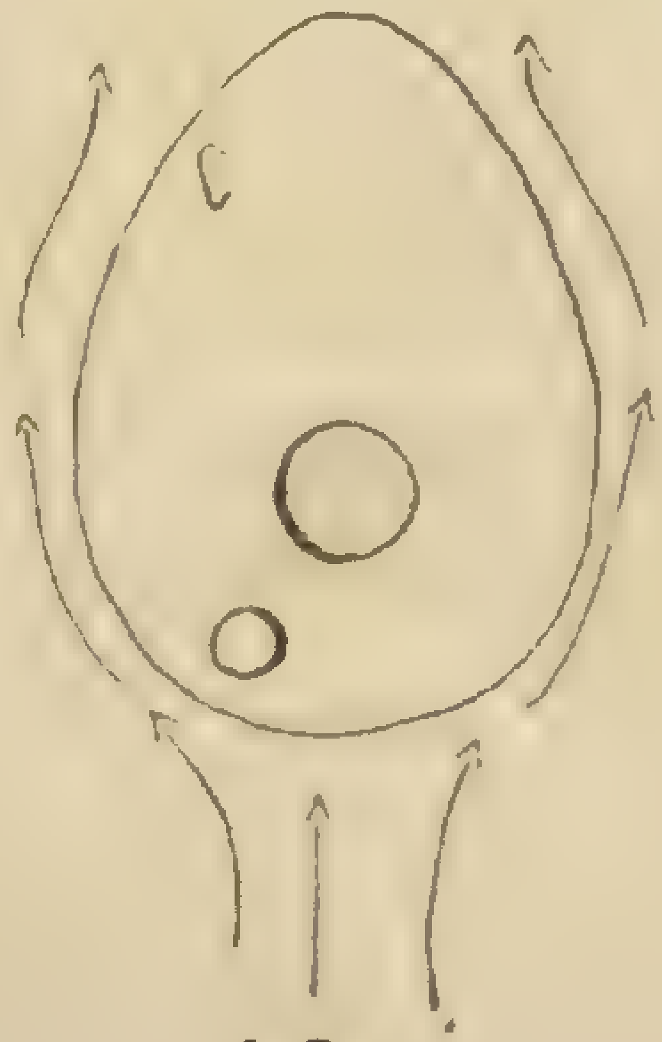

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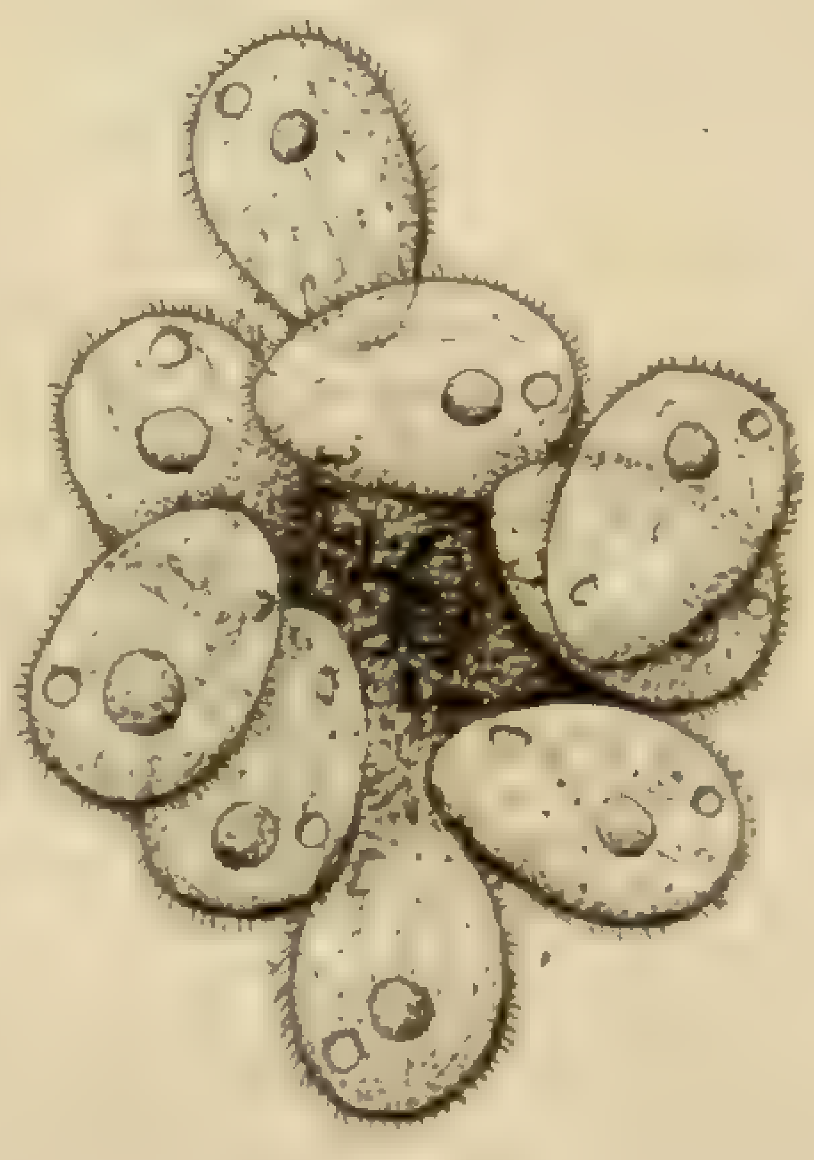

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rapidity for a few seconds, then flutter slowly, then close for a second or two, and then begin slowly to beat again. From experiments which have been made the conclusion is drawn that the rate of the beating of the oral membranes in *Glaucoma* is directly proportional to the strength of the food stimulus received in the buccal cavity, or possibly through the cilia in the region of the mouth.

The lips do not open and close with their ectal edges parallel. The process of closing takes place with a wave-like motion, the anterior edges of the lips meeting first, and the wave of motion thus initiated traveling toward the posterior ends, until, when these finally meet, the anterior edges are open again. The process of opening, therefore, takes place in the reverse direction. Fig. 8 diagrams six stages in a single beat of the lips, and in Fig. 9 are represented four beats analyzed with respect to their cycles of contacts and partings.

The opening and closing of the inner lips, or edges of the mouth proper, take place, apparently, in a similar wave-like manner, and in the reverse order or motion from that of the outer lips. This sequence of motion may aid in forcing food into the buccal cavity, since whenever any given portion of the buccal cavity is open

FIG. 8. Six stages in a cycle of the opening and closing of the outer lips. The edges of the lips are represented diagrammatically to show how the waves of contact and parting follow each other. Stage 7 begins the cycle anew.

FIG. 9. Graphic representation of the relations of the cycles of opening and closing of the outer lips. Four beats are analyzed, each of one half second's duration. The vertical lines represent the line of the outer edges of the lips, and the lines of crosses and circles show how the waves of contact and parting sweep up and down them. Four stages are given for each beat—represented by the four vertical lines of the lips in each beat. The longer vertical lines represent the stages when no part of the lips are in contact. Stages 1, 2, 3, 4, 5, in this figure correspond respectively to stages 1, 2, 4, 5, 6, in Fig. 8.

FIG. 10. Five stages of the cycles of opening and closing of both the outer and inner lips diagrammatically represented, and superimposed to show their reverse relationships. The *o*-lines represent the edges of the outer lips, and the *x* lines the edges of the inner lips.

FIGS. 11, 12, and 13 show the various directions from which water currents, bearing food, can be urged towards an individual by the action of the cilia.

FIG. 14. Group of *Glaucoma* clustered about a mass of debris and bacteria, feeding.

to receive food, the corresponding portion of the lips above is closed, to force it in. Fig. 10 shows diagrammatically the relationship of the cycles of opening and closing of the outer and inner lips. It will be noted that once during each cycle (at its middle) both the outer and inner lips correspond, for an instant, in attitude, the same portions of each being in contact (Stage 3, Fig. 10). In some individuals the inner lips seemed not to open and close at all, but appear to remain permanently open. Observations on the motions of the lips were made while the animals were incarcerated in very thick gelatine, and all of their movements much retarded.

The ciliary action of the anterior portion of the body can apparently be regulated according to the desires of the animal and water currents, bearing food, can be drawn toward the mouth either from directly in front of the creature, from the side, or from behind (Figs. 11, 12, and 13).

*Glaucoma* feeds upon minute flagellates and other minute protozoans, fragments of débris, tiny diatoms and desmids, but the bulk of its food seems to consist of bacteria. Fig. 14 shows what may frequently be observed in a culture, *i.e.,* a group of individuals clustered about a mass composed mainly of bacteria, pressing their lips against the substance, and urging portions of it into the buccal cavity by the combined action of the lips, and oral cilia. When feeding in this fashion the movements of the outer lips resembles somewhat the biting and chewing motions of the mouths of the higher animals.

Curiously enough the complex development of the exterior oral appendages seems to have no parallel within the buccal cavity, since neither supporting pharyngeal rods, nor even any definite pharynx, could be discerned.

# NOTES ON THE BIONOMICS OF MELLITA[1]

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OBSERVATIONS already recorded[2] led me to devote some attention to the movements and the coloration of *Mellita sexies-perforata,* and to the injuries which are in nature inflicted upon the individuals of this species. The findings incidentally supplement and confirm some deductions made in the notes cited,[2] and serve to indicate several directions in which further study would seem promising.

*Mellita* lives more or less completely buried in the sand, in channels between islands, at the outlets of sounds, and between the shore and the inner reefs; but always in places where there is a tidal current. The character of the bottom, which may lie 2 to 6 fathoms beneath low water, varies from shell-sand, gray-white and usually muddy, to dark brownish mud. Young and adults of all sizes up to 13 cm. transverse diameter occur in company. The older individuals burrow more deeply than the young ones, the latter frequently lying freely exposed on the surface of the sand. In stormy weather, all the Mellitas dig themselves deep into the mud, to a depth of perhaps 8–9 cm. In the laboratory the older individuals burrow more quickly when exposed to bright sunlight than they do in the dark. Heliotropism, when horizontal light is used, is not precise; younger individuals tend, on the whole, to move away from a source of horizontal light. Normally, light coming from above may be a significant source of stimulation; this was not adequately tested. The low degree of photic irritability toward horizontal light probably accounts for the fact that no photic orientation, strictly speaking, was noted; such orientation might be expected, since the nature of Mellita's locomotion would make it possible.

In locomotion on a solid surface, that part of the body anatomically the anterior is always carried ahead. The "leading"

[1] Contribution from the Bermuda Biological Station for Research, No. 118.

[2] Crozier, W. J., 1918, "On the pigmentation of a Clypeastroid, *Mellita sesquiperforatus* Leske," AMER. NAT., 52, 552–555. 1919, "On Regeneration and the Re-formation of Lunules in *Mellita,*" AMER. NAT., 53, 93–96.

point may shift progressively from side to side during an extended act of creeping, but *at all times* some part within the two anterior interradii is in advance. *Mellita* can, however, pivot in complete circles, in either direction, about its mouth as a center; it also carries out successive incomplete swings, alternately opposite in direction. Movements of the latter type, together with the relatively fixed direction of creeping, namely anteriorly, are important for the act of burrowing, and are correlated with some notable growth-changes in the form of the whole body.

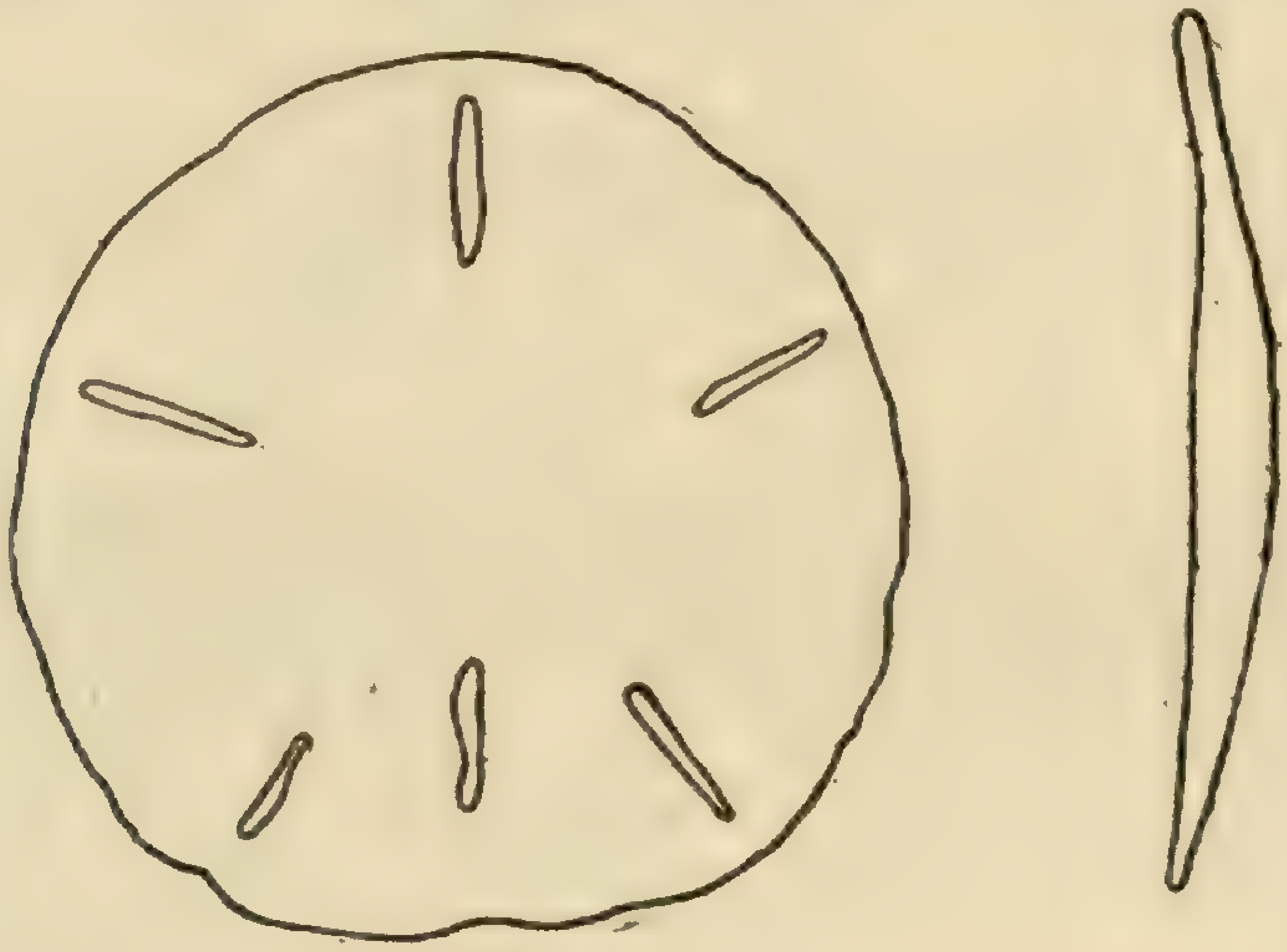
During burrowing the anterior end is in advance. The process of concealment is a fairly rapid one, a large specimen being able to disappear completely in less than 15 minutes, although two to three times this interval may be employed. Not only do the spines and tube feet assist in clearing a way through the sand, by moving the individual sand grains, but, in addition, the body as a whole is used as a digging instrument. The disk is repeatedly rotated 30° or more to either side of the sagittal line,

FIG. 1. Outlines of three young *Mellita sexies-perforata*, showing the approximately circular outlines of the body; between the specimens of intermediate and largest size is an antero-posterior section of the former. Attention may be called to the persistence of the marginal notches opposite the two posterior ambulacral lunules, although these lunules, in *M. sexies-perforata*, are formed by the meeting of dorsal and ventral invaginations, not by the inclusion of reëntrant marginal notches—as is the case in other *Mellitas*. (× 1.)

with the result that, since the very numerous spines and tube feet are simultaneously pressing the disk forward, the animal is actually insinuated, or "slid," into the ground. This maneuver is especially effective upon a muddy bottom, where movement of sand by the tube feet and spines would be a slow and inefficient process.

The young *Mellita* is quite thin and wafer-like, its outline practically circular (Fig. 1). As the animal grows, the thickness of the body increases, although the edge of the disk remains thin. In many cases the outline of the disk is still almost cir-

cular even in specimens of maximal size, and the ventral surface of the disk practically flat (Fig. 2). It frequently happens, however, that the central region of the body becomes relatively thicker than in the flat, circular sea-plates, and in these individuals there are several noteworthy departures from the typical structure. Anteriorly the disk is more wedge-shaped in vertical



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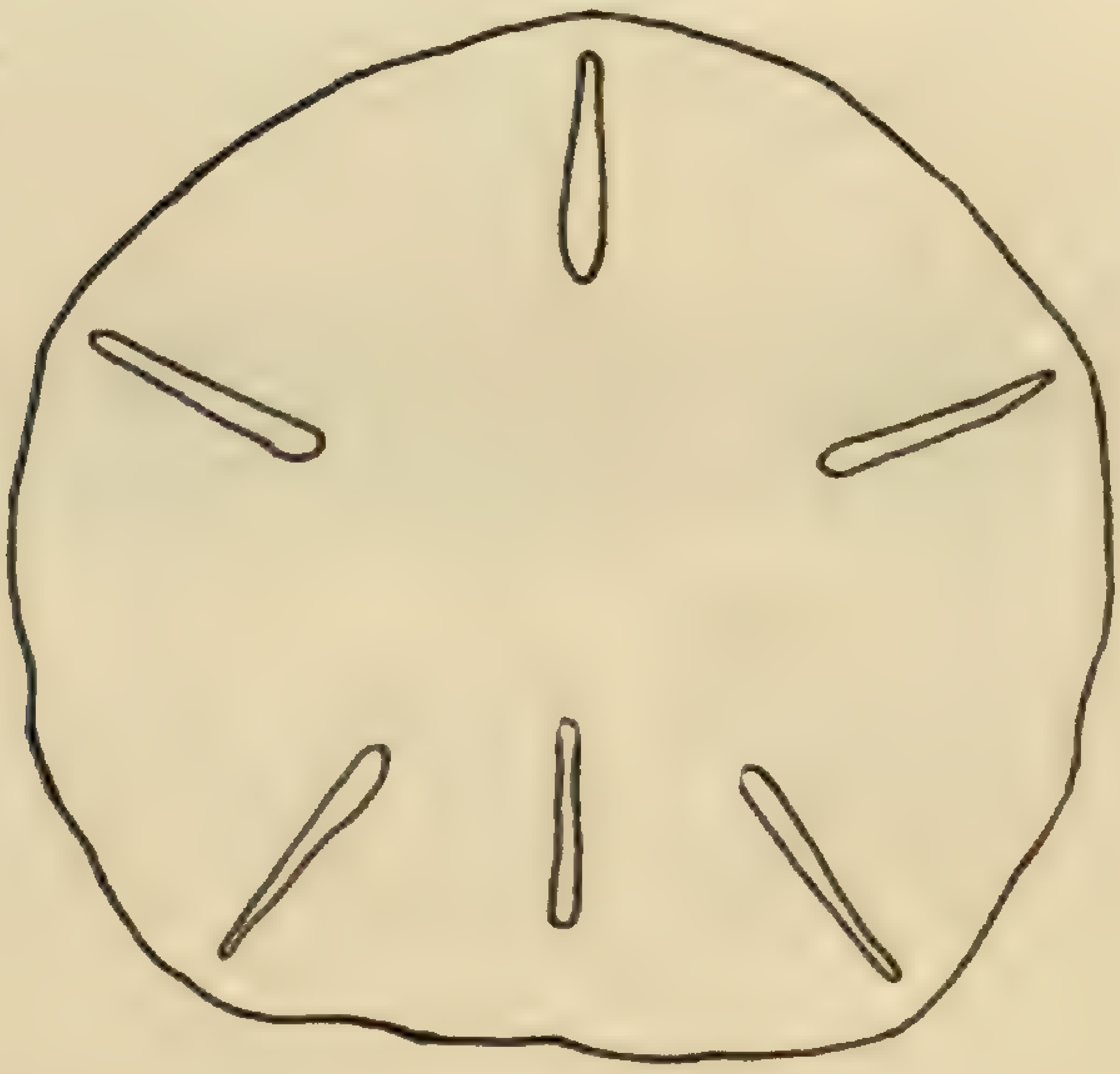

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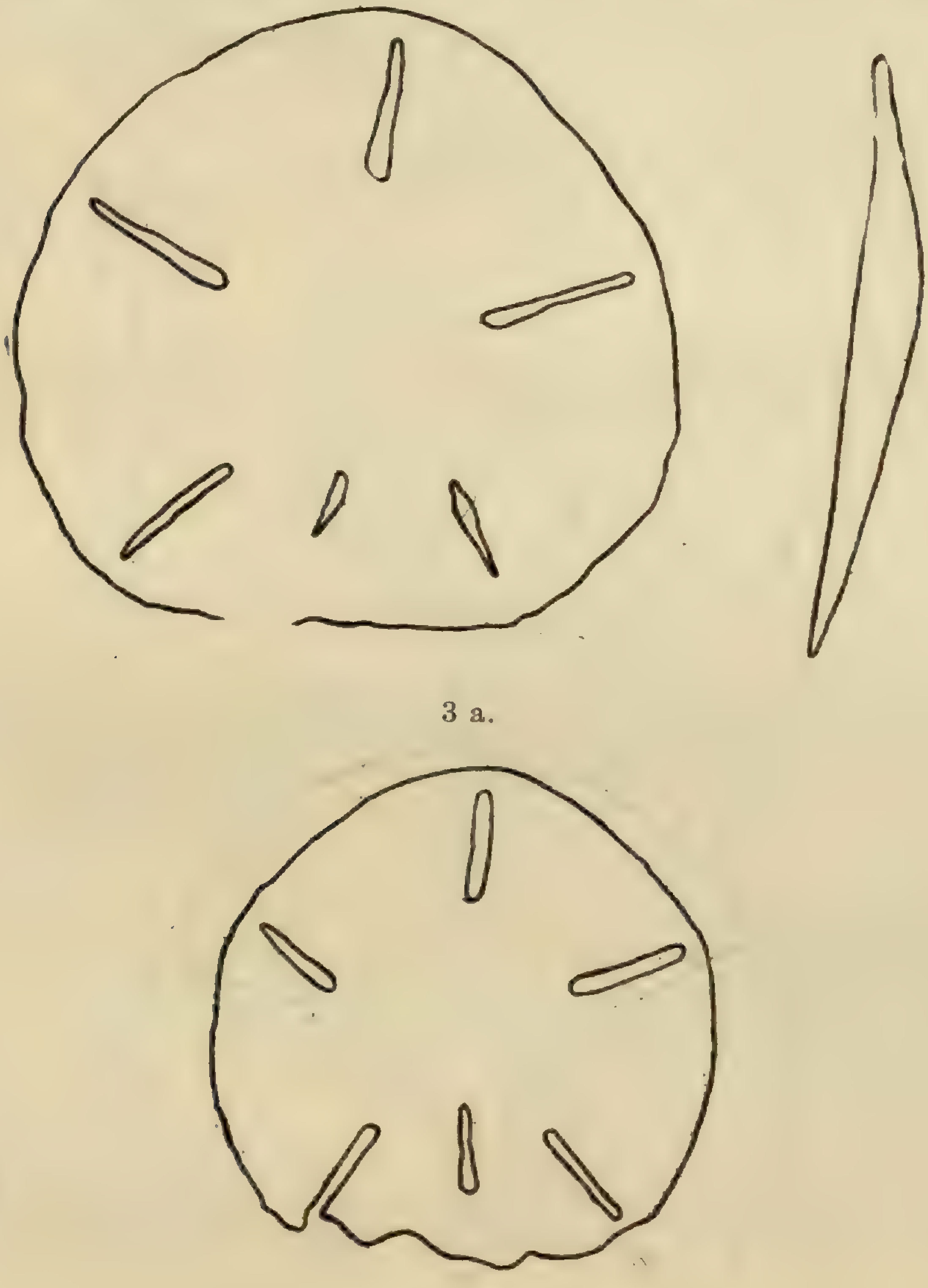
Fig. 2. Outlines of two individuals with circular type of margin, with a sagittal section of one of them (*A*). ($\times 2\frac{1}{2}$.) In this section, and in the following ones, it will be observed that the anterior margin of the disc is more bluntly rounded than that at the posterior edge—an advantage, presumably, since the burrowing portion of the periphery is thus made stronger.

section; the anterior antimere projects forward, forming a sharp "nose," or entering point, which presumably facilitates burrowing (Fig. $3^{A}$). The antero-lateral radius on either side

sometimes forms, in addition, a more or less projecting "shoulder" (Fig. 4). These departures from the smoothly circular form, together with the "arched" construction of the test in some older specimens (Fig. 4), derive their effectiveness for



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3 b.

FIG. 3. Outlines of two individuals with projecting anterior radii, and a sagittal section of one of them. (× ½.)

burrowing from the partial rotation or "swinging" of the disk during this act.

The changes here noted in the form of the body of some individuals with advancing growth, are not detectably correlated with peculiarities of habitat. The different types occur with about the same relative frequency whether the bottom is of shell sand or of brownish mud. The local character of the bottom

changes somewhat, however, from time to time, being more muddy and less sandy in some years than in others. One may nevertheless entertain the idea that genetic factors are concerned in determining the growth-changes in the body-shape of some individuals. This matter should be studied in a larger series of specimens than I have been able to secure in the time devoted to this work. Especially interesting is the fact that this

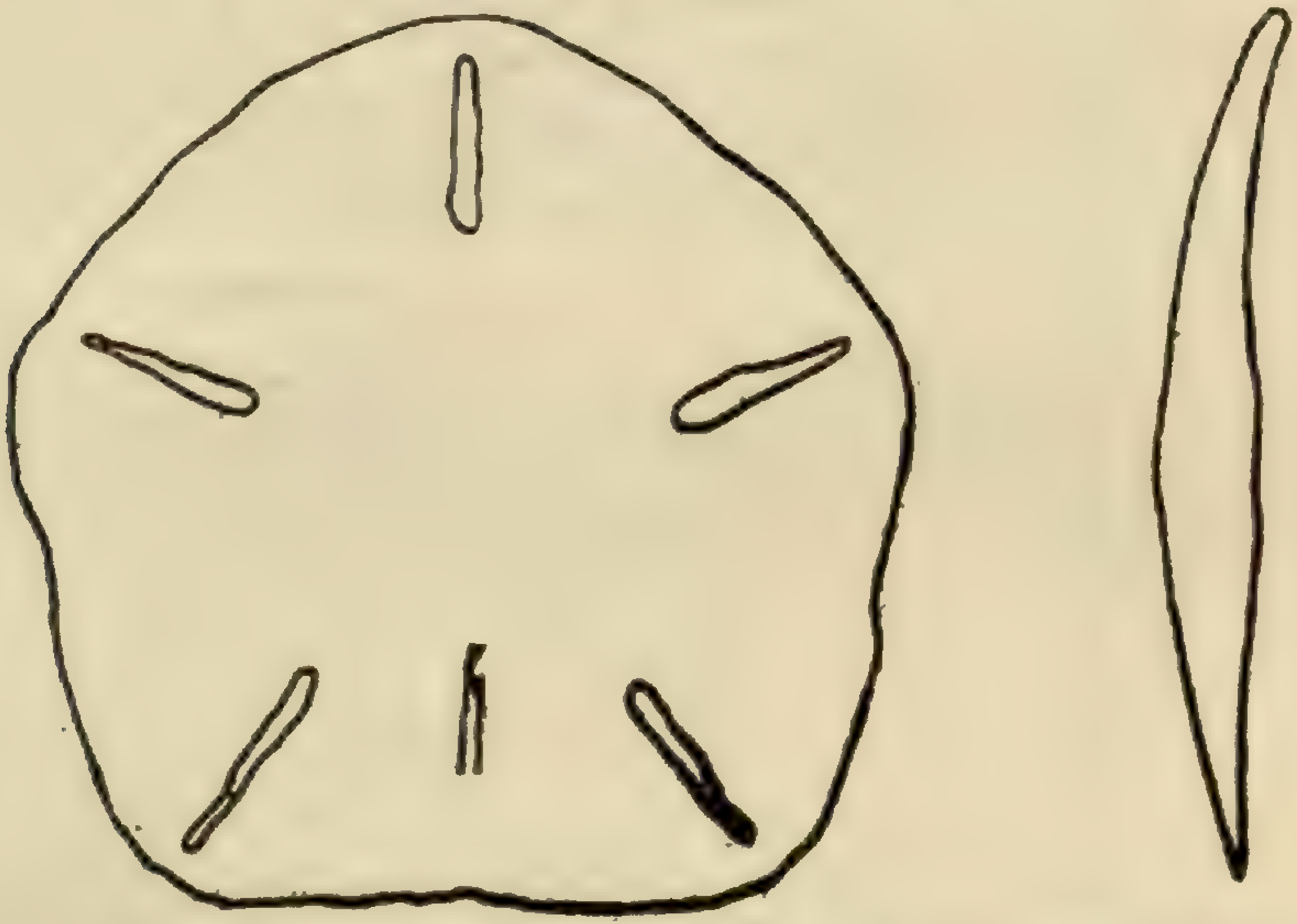

FIG. 4. Outline and sagittal section of a *Mellita* pointed anteriorly and with lateral "shoulders"; the section shows the tendency toward an arching of the test in some of these cases.

species occurs in the fossilized state above the beach zone along the shores of islands in Great Sound. The seven large fossil tests I have examined were all of the almost perfectly circular type (cf. Fig. 1).

In order to follow more precisely the course of growth-changes in *Mellita,* I endeavored to derive the curve of its growth, and to fix the average duration of its life. The results, which are provisional only, are given in Fig. 5. Specimens were measured from one locality—Cobbler's Cut, Spanish Point—in September. The *transverse* diameter was measured, since it is less subject than is the antero-posterior to fluctuations induced (a) by the tendency, already noted, to form a projection at the anterior antimere, and (b) through injuries suffered at the posterior inter-radius. From the modes in the frequency distribution of sizes, it was deduced that at 6-months *M. sexies-perforata* measures 2.2 cm. in transverse diameter; at 1.5 years, 5.7 cm.; and so on, as indicated in Fig. 5. It seems possible that the average duration of life is about 4 years, according with the fact that

the majority of the dead tests, which may be collected in quantity, are about 10 cm. in transverse diameter. From these estimates of age in *Mellita*, the described growth-changes in the form of the body begin to become obvious during the third year of an individual's life; in some specimens they do not occur at all.

During the middle years of its life, *Mellita* has a considerable capacity of withstanding injuries (cf. Fig. 6) and of repairing damage done to the periphery of its body. I have already noted the fact that injured and regenerating individuals are found to have been damaged at the *posterior* end only; the wound illus-

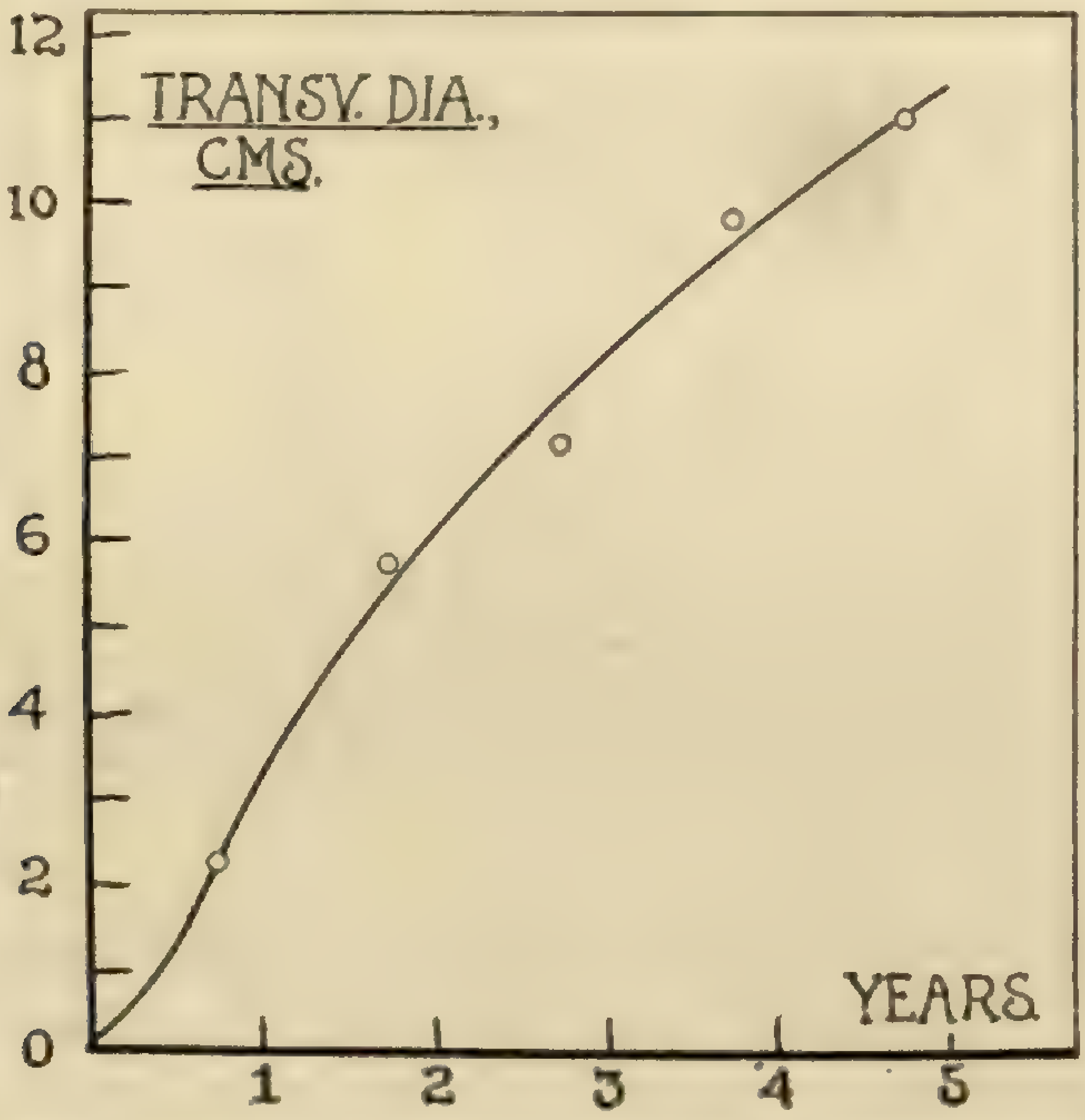


FIG. 5. Possible growth curve of M. *sexies-perforata.*

trated in Fig. 6 is unusually far forward. It was previously suggested (Crozier, 1919) that the posterior incidence of injuries resulted from the circumstance that the posterior end was more freely exposed than the rest of the body. Observations in aquaria and on sand-beaches have shown this explanation to be probably correct. Burrowing takes place with the anterior end in advance, the edge of the posterior inter-ambulacral area frequently remaining exposed long after the rest of the creature has been concealed beneath the sand. The experimental tests were made with active, healthy, specimens exhibiting no green areas upon their surface (cf. Crozier, 1918). During burrowing, the body of the sea-plate is moreover tilted, anterior end down, at an angle of 10°–20° with the surface of the sand; so

that the exposed posterior or postero-lateral margin projects in a not inconspicuous way above the general level of the sand.

In the light of this behavior, and particularly because of the injuries found to have been suffered by the sea-plates, it becomes pertinent to inquire whether an adaptive (concealing) value attaches to the pigmentation of these animals. The occurrence of injured specimens, and that in some degree of frequency (about 60 per cent. of those above 9 cm. transverse diameter), would seem in itself to be valuable evidence upon this point. There are several other important considerations to be derived from the nature of the pigmentation of *Mellita.*

Until it has attained a diameter of 7 to 8 cm., the young *M. sexies-perforata,* seen from above, is practically colorless; the integument contains no pigment, although the yellow-brown stomach may show faintly through the test. Upon attaining this size, a light coffee-tint, evenly distributed upon the dorsum, makes its appearance; previously, at about 5.7 cm. diameter, dark brown pigment begins to show on the *ventral* surface, on each of the polygonal areas surrounded by the tube-foot channels. Pigment thus begins to be deposited on the ventral side; and it continues to be denser (darker) on this side than dorsally. The intensity of pigmentation increases progressively with age, until, in animals on 12–13 cm. diameter, a very dark brown hue is attained.

FIG. 6. Illustrating natural injury and initial stages of repair. From the living animal. (× ½.)

*M. sexies-perforata,* at Bermuda, does not frequent bottoms supporting a good growth of eel-grass. If in other regions it should be found to do so, the alkali-greening substance occurring in this species (Crozier, 1918) and in Clypeastroids generally, might be adaptively concerned in pigmentation. But no green hues are normally evidenced by this species at Bermuda. If the somewhat uncertain records of a green coloration in normal mellitas of this species at more southerly stations are confirmed, and found related to an eel-grass habitat, a direct physical explanation is at hand to account for the greening (cf. Crozier, 1918). The normal brown hue seems due to the integu-

mentary accumulation of some metabolic waste. This view in itself does not preclude the possibility of an adaptive determination of the pigmentation; it would be curious indeed if an internal coloring-matter were not the result of metabolism. In order to remove the possibility of an explanation for the coloration in the customary terms of adaptation, it must be shown that the pigmentation in question is an *unconditioned* result of metabolic processes—unconditioned, that is, by the "need" for concealment and the like. It is, therefore, important to observe that: (1) the degree of pigmentation increases with age; (2) the variously colored individuals live side by side; (3) the tilting of the body exhibited during burrowing, and the exposure of the posterior margin of the body owing to the incompleteness of this act, are not compensated by counter shading—the ventral surface is more darkly colored than the dorsal; and (4), the region known to be differentially exposed in this way is found actually to bear evidence of damage, in a goodly proportion of individuals. The exact origin of these injuries remains obscure, but is not of primary importance here.[3]

The general physiology of pigmentation in the sand-dollars and sea-plates, and the possible evolutionary significance of the growth changes in body-form noted in this paper, should be made the topics of further studies.

Dyer Island, Bermuda, 1918.

[3] I have good reason to believe that the injured mellitas were not damaged as the result of antecedent dredging operations in the same areas.

# THE INSECT ENEMIES OF POLYPOROID FUNGI

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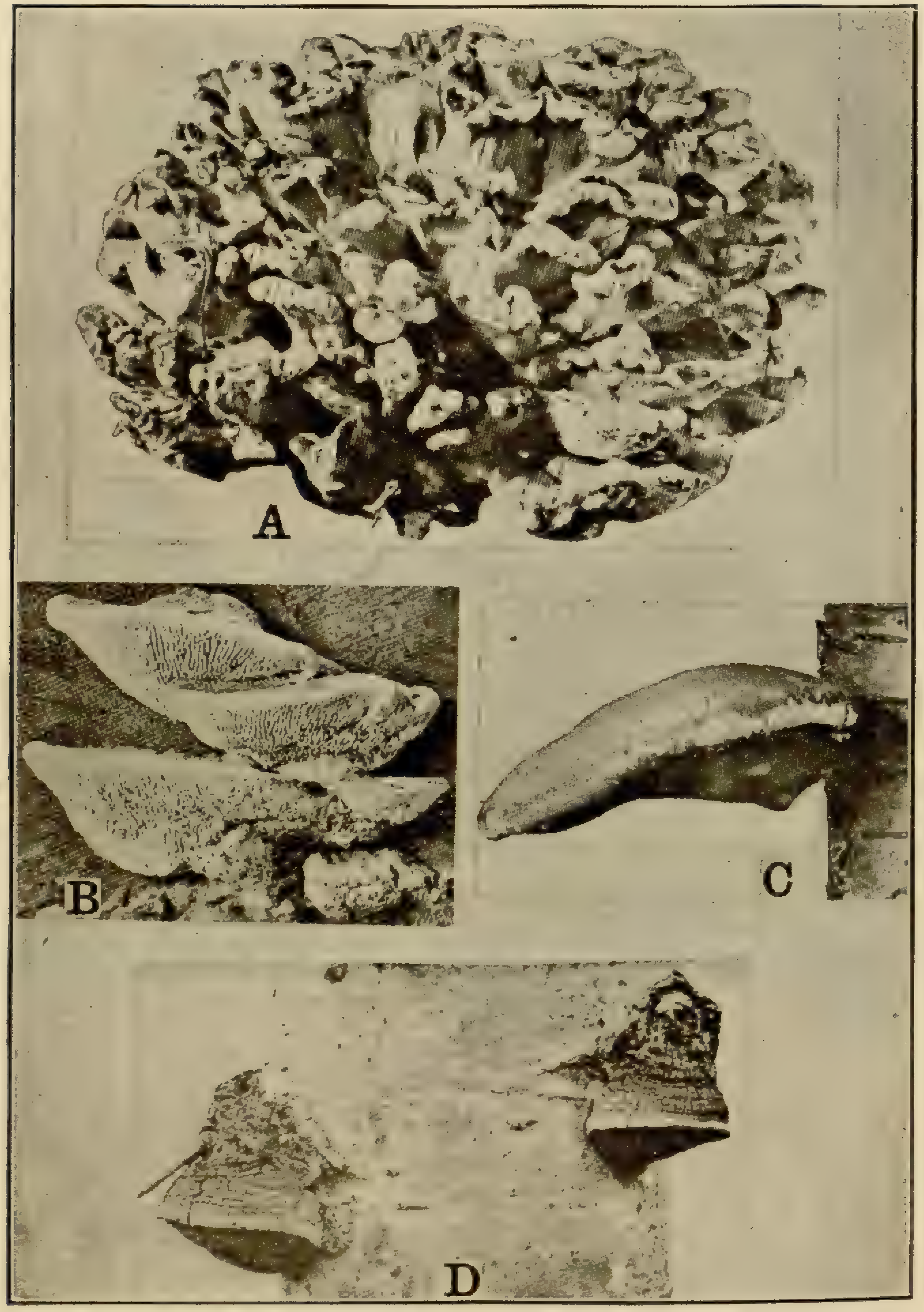
IN the past, entomologists have paid little or no attention to the fungus hosts from which they collected insects, and their captures are usually recorded as having been taken on or in a "fungus." This is a very indefinite term which includes a large number of species and gives no clue whatever as to the identity of the host. Years ago when mycologists were few and far between, it was undoubtedly difficult for entomologists to have the fungi identified but at present this excuse will no longer hold and there is no reason why such hosts should not be specified.

What appears to be a definite relationship between certain fungi or their fruiting bodies and insects has been observed in the past by both entomologists and mycologists. The spores of certain fungi were found to germinate in certain insect burrows and infection in some cases took place through insect apertures. In other cases, certain insects were observed to be present with certain fungi, both on a common host. Whether they acted together, independently, or followed each other in connection with the death of the host is a subject for further study.

The main object of this paper is to call attention to the different groups of insects which are found associated with polyporoid fungi and to urge that the hosts be recorded specifically or as near that as possible so that future workers will be in a position to digest the information intelligently after a large enough mass has accumulated. This paper is really a summary of the observations made during a year's collecting of fungus insects in New Jersey.

The *Polyporaceæ* includes those forms in which "the hymeneal surface is generally spread over the inner surfaces of pores or narrow tubes, sometimes over folds or shallow depressions between vein-like reticulations occasionally more or less lamelloid."[1] The sporophores vary considerably, are often very large and usually tough, and found chiefly on wood in the form of brackets of various sizes and shapes. The members of this family are found on both living and dead wood of deciduous and co-

[1] Duggar, B. M., "Fungous Diseases of Plants."




*A*. Fruiting bodies of *Polyporus sulphureus*.

*B*. Sporophores of *Dædalia quercina* on oak railroad tie; rarely attacked by insects.

*C*. Sporophore of *Polyporus betulinus* on birch.

*D*. Sporphores of *Fomes igniarius* on aspen.

(All figures after Von Schrenk and Spaulding, Bul. 149, U. S. D. A. Bur. Pl. Indus.)

niferous trees and as a class are very destructive to trees and timber.

New Jersey is not particularly rich in this group, only some 50 species having been found up to the present time and the state has been fairly well collected over. Many of these were taken in what is known as the Piedmont Plain section and while this area is largely under cultivation, it has many large swamp areas and the forests are deciduous. The Pine Barren section of the state was almost devoid of polypores and in the other sections they were found in carying numbers, depending on the size and location of the forested area. Several sections of the Piedmont Plain yielded the most and the species were by far more numerous here than in any of the other sections of the state.

Of the 50 different species of polypores collected 80 per cent. were found to be infested by insects, which were distributed as follows: Of a total number of 74 species, 59 belonged to the Coleoptera, 1 to the Hemiptera, 3 to the Lepidoptera, 5 to the Hymenoptera and 6 to the Diptera. Of the 6 species of Diptera, 2 were crane flies found associated with soft, watery polypores, 3 were fungus gnats and 1 was a member of the *Ortalidæ.* All of the Hymenoptera were parasites of beetles. The 3 species of Lepidoptera belonged to the family *Tineidæ* and the one hemipteron was the flat bug *Aradus similis* found on *Polyporus betulinus* and *Fomes pinicola.* In the Coleoptera 17 families were represented, according to the following table:

COLEOPTERA ASSOCIATED WITH POLYPORES

| Family | Number of Species Found on or in Polypores |
|---|---|
| Hydrophilidæ | 1 |
| Staphylinidæ | 3 |
| Scaphidiidæ | 2 |
| Erotylidæ | 4 |
| Mycetophagidæ | 3 |
| Dermestidæ | 1 |
| Histeridæ | 3 |
| Nitidulidæ | 6 |
| Trogositidæ | 2 |
| Ptinidæ | 3 |
| Bostrychidæ | 1 |
| Cisidæ | 16 |
| Scarabaeidæ | 2 |
| Tenebrionidæ | 6 |
| Melandryidæ | 4 |
| Mordellidæ | 1 |
| Anthribidæ | 1 |
| | 59 |

With the exception of the species belonging to the *Histeridæ* and part of the *Trogositidæ,* which are predaceous, it is extremely probable that most of the remainder are fungus eaters, as some were taken breeding in and others on or in the fungi. In addition to what might be called the fungus habit of some of the members of the above named families, it might be of interest to note other family habits in a general way and this can be presented best in a tabular form.

OTHER FAMILY HABITS OF COLEOPTERA FOUND IN POLYPORES

| Family | General Habits of Members |
|---|---|
| Hydrophilidæ | Aquatic and terrestrial, found in moist earth, dung, decaying vegetation. |
| Staphylinidæ | Varied, predaceous, feeders on decayed vegetation. |
| Scaphidiidæ | Living in rotten wood, gill fungi. |
| Erotylidæ | In fungoid growths, stems of plants. |
| Mycetophagidæ | Under bark and in fungus. |
| Dermestidæ | On dry animal matter and vegetable products. |
| Histeridæ | Predaceous. |
| Nitidulidæ | Varied, sap beetles, in fungi or dry animal and vegetable matter. |
| Trogositidæ | Predaceous, a few in granaries, fungi. |
| Ptinidæ | On dry animal and vegetable products. |
| Bostrychidæ | In dry wood. |
| Cisidæ | In fungi or wood penetrated by fungi. |
| Scarabaeidæ | Varied, in excrement, decaying vegetation, etc. |
| Tenebrionidæ | Scavengers, on dead or dry wood, vegetable products, in granaries. |
| Melandryidæ | In dry vegetable matter. |
| Mordellidæ | On flowers, dead trees, in plant stems. |
| Anthribidæ | On dead wood, on fungi. |

The members of the *Cisidæ* and *Mycetophagidæ* appear to be the only ones confined almost exclusively to fungi. It is quite probable, however, that such members of the other families as are listed as occurring under bark and in dead wood are feeders on the fungus hyphae which penetrate such places.

Many of the polypores, especially after they have matured, are dry, tough, woody or leathery, although some are soft and watery. In most cases the entire sporophore is consumed by the beetles and their larvæ, or so riddled that it weathers away rapidly. In many instances the partly eaten sporophore affords hibernation quarters for the larvæ or adults during the winter and food the next season until fresh fruiting bodies are produced.

Certain species of polypores appear to be more attractive to insects than others and the following table gives a list of the fungi, together with the numbers of insects in different orders found associated with each fungus.

POLYPORES AND INSECTS FOUND ASSOCIATED WITH THEM

| Fungus | Coleoptera No. Species | Lepidoptera No. Species | Diptera No. Species | Hemiptera No. Species | Total |
|---|---|---|---|---|---|
| *Polyporus squamosus* Hud. | 1 | | | | 1 |
| " *brumalis* Pr. | 1 | | | | 1 |
| " *betulinus* Bul. | 8 | | | 1 | 9 |
| " *berkleyi* Fr. | 11 | | | | 11 |
| " *sulphureus* Bul. | 2 | 1 | | | 3 |
| " *fumosus* Per. | 2 | | | | 2 |
| " *amorphus* Fr. | 2 | | | | 2 |
| " *conchifer* Schw. | 1 | | | | 1 |
| " *tulipiferus* Schw. | | 1 | | | 1 |
| " *pargamenus* Fr. | 3 | | | | 3 |
| " *versicolor* L. | 23 | | | | 23 |
| " *hirsutus* Wf. | 5 | | 1 | | 6 |
| " *dichrous* Fr. | 4 | | 2 | | 6 |
| " *borealis* Fr. | | 1 | | | 1 |
| " *chioneus* Fr. | 2 | | | | 2 |
| " *albellus* Peck | 6 | | 1 | | 7 |
| " *galactinus* Peck | 1 | | | | 1 |
| " *cinnabarinus* Jacq. | 3 | | | | 3 |
| " *lucidus* Leys. | 3 | | 1 | | 4 |
| " *curtisi* Berk. | 1 | | | | 1 |
| " *tsugæ* Mur. | 3 | 1 | 1 | | 5 |
| " *graveolens* Schw. | 1 | | | | 1 |
| " *hispidus* Bul. | 1 | | | | 1 |
| " *gilvus* Schw. | 10 | | | | 10 |
| " *cuticularis* Bul. | 4 | | | | 4 |
| *Fomes pinicola* Swen. | 1 | | | 1 | 2 |
| " *bakeri* Mur. | 1 | | | | 1 |
| " *fomentarius* L. | 1 | | | | 1 |
| " *igniarius* L. | 2 | 1 | | | 3 |
| " *lobatus* Schw. | 1 | | | | 1 |
| " *applanatus* Per. | 3 | | | | 3 |
| " *marmoratus* | 1 | | | | 1 |
| *Trametes suaveolens* L. | 1 | | | | 1 |
| *Dædalia unicolor* Bul. | 1 | | | | 1 |
| " *confragosa* Bol. | 3 | | | | 3 |
| " *quercina* L. | 1 | | | | 1 |
| *Lenzites betulina* L. | 8 | 1 | 2 | | 11 |
| " *sæpiaria* Fr. | 2 | | 1 | | 3 |
| *Poria* Sp. | 2 | | | | 2 |

From this table it will be seen that *Polyporus versicolor*, *Polyporus berkleyi*, *Polyporus betulinus*, *Polyporus gilvus* and *Lenzites betulina* are all insect favorites, especially *Polyporus versicolor* with 23 species to its credit. Just why these species are so attractive to the Coleoptera is a subject for further study. In fact any discussion at this time of the relationship between insects and fleshy fungi would be unwise on account of a lack of suitable data and the above material has been presented in the hope that it may stimulate interest in a subject which has long been neglected and which contains interesting and perhaps economic possibilities.

# SHORTER ARTICLES AND DISCUSSION

## NOTES ON THE NEMATODE GENUS CAMALLANUS

RECENTLY a paper by Dr. G. A. MacCallum entitled "Notes on the Genus *Camallanus* and Other Nematodes from Various Hosts" has come to my attention. It is dated July, 1918, and appeared in *Zoopathologica.* I have carefully investigated the date of the paper and find that although it appeared in the July, 1918, number of *Zoopathologica* it was not received at the Library of the Bureau of Animal Industry until August, 1919, and at that time neither the John Crerar, the National Museum Library, the Library of Congress, nor the New York Public Library had received their copies. The Secretary of the New York Zoological Society stated that the edition was sent to Dr. MacCallum, October 14, 1918, and as nearly as I can learn was not mailed out until August, 1919. In a personal letter Dr. MacCallum stated that the paper was "partly issued on July 1, 1918." My paper on *Camallanus americanus* was mailed out on August 13, 1919, and since I can not obtain a definite statement as to the date on which MacCallum's paper was mailed, it is impossible for me to state which paper actually has priority of date. MacCallum's paper was unknown to me at the time my monograph on *C. americanus* (1919) was published; hence it seems that I should discuss the form described by MacCallum, since some of my concepts concerning the genus and its species do not conform with his observations.

In the first place, attention should be called to the fact that MacCallum was undoubtedly misled by the brevity of a preliminary paper by Ward and Magath (1917) into thinking that we believed the two species described were the first from America, as MacCallum asserts. We were well aware of the fact that Leidy (1851) had found the genus, under another name, many years before and had named several species from America. At the time we had many records of it and a great deal of material and, in fact, considered it a common parasite. I have gone over the paper quite carefully and can not see why MacCallum should say that the "general tenor" of our paper implies that the worm is rare.

MacCallum apparently places the genus *Camallanus* in a sub-

family within the family Strongylidæ; I am sorry to disagree with him, however, on fundamentals. He bases his argument on four statements:

1. These worms live in the same organ. However, so do many other worms, trematodes, cestodes, and so forth.

2. They have identical habits and attach themselves to the mucous membrane in the same manner. The true situation is clearly set forth in Looss's (1905) monograph on the hookworm and in my paper on *Camallanus americanus.* Ward (1917) has called attention to the fundamental structural difference in the buccal apparatus of the Strongylidæ and I have pointed out the various methods of attachment to the mucous membrane. The members of the genus *Camallanus* are blood suckers; this may be true of some strongyles, but not of the hookworm. Looss has shown clearly that it is not a blood sucker and gets blood only incidentally as it feeds on the mucous membrane of the host.

3. The muscular pharynx or upper part of the esophagus is essentially the same in both types. This part of the esophagus is the same in all the Myosyringata.

4. The structure of their mouths MacCallum believes to be essentially the same. Ward and I have called attention to the basic fact that the mouths of the strongyles are built up on the circular plan, that they hold fast by suction and then shave off the mucous membrane with additional structures, such as internal cutting plates of the hookworms. In the genus *Camallanus* the mouth is built up on a lateral plan. The valves are in truth jaws and bite; suction is secondary and is produced, as in all Myosyringata, by the action of the esophagus.

It would lead me too far astray to take up all the differences between the families Camallanidæ and Strongylidæ because they even belong in different superfamilies, but I may point out the fact that the former are viviparous forms, while the latter are oviparous, that the Camallanidæ have but one single ovary and the males genital alæ, while in the other family the females have two ovaries and the males have bursæ. These are such important facts in the structure of nematodes that were there no other points of difference, such as the double character of the esophagus in the Camallanidæ, the heavier character of the spicula, and the lack of gubernacula, that they could not be considered in the same subdivision.

I must call the reader's attention to the following points in MacCallum's brief description of the genus *Camallanus:*

1. The length of the worms as given does not embrace enough variation, nor does the recorded width. Worms within this genus have been recorded nearly twice the greatest dimensions which he gives, and mature females have been found much smaller than those which he describes.

2. The ridges in the valves are not always "six main ribs on each side of the central line."

3. My experience is that the worms are dislocated from the mucous membrane with difficulty and that often they are pulled in two or a plug pulled out of the intestine before they can be loosened.

4. I presume that "the bar of chitin across the anterior ends of the median four" ridges is the structure that I have called the anterior wing and its purpose is the attachment of the giant buccal muscles primarily and not for "stiffening them" only, if at all.

5. Certainly not all the males have "three pairs of post-anal papillæ, and one pair of pre-anal." In fact, I am sure that most if not all members of this genus have seven pairs of pre-anal papillæ and five pairs of post-anals, with two para-anal pairs.

6. "The semicircular flap" does not act like a bursa. The action of this structure, correctly called "alæ" has been explained by Magath (1919) and its difference from the action of a true bursa shown.

With regard to the new species; MacCallum identified *C. oxycephalus* from a Mississippi alligator. It is impossible to know whether or not he really had a worm belonging to this species. It would be interesting to know that a species harbored in fishes could live in an alligator. If, as he says, the "general description" from the genus will answer for this species he can not be certain that he had *C. oxycephalus* because there is nothing in his general description that is specific for this worm or any other species.

MacCallum's descriptions of *C. scabræ* and *C. troosti* are so indefinite and meager that either species can not be identified positively from the descriptions and figures. He gives very few measurements and the magnification of the figures is not stated. I had hoped to be able to compare his species with *C. americanus* but I can find no definite data in his paper on which to base the comparison.

The description of *C. chelydræ* is passed over with the state-

ment that "there is no appreciable difference between these and the general description" of the genus which MacCallum states also fits *C. oxycephalus.* The status of this new species seems to me to be doubtful.

MacCallum's basis for describing *C. floridianæ* as new seems to lie in the fact that

> the rods are placed on two levels, one set of four being in front and the other set of four placed behind, and diagonally across the first. Whether this condition is only an exemplification of the way in which they act normally by a sawing motion, I do not know, but this would be reasonable. They may act like a pair of hair clippers and were caught in this position when death took place.

The action which MacCallum assumes is probably not the method of action of these jaws. Since I have previously pointed out the method of action of the jaws, no more needs to be said here on the subject. The criss-cross arrangement of the ridges in the mouth apparatus is well explained on the basis of MacCallum's drawing, which shows that the head of the worm was tilted; I have often seen the same condition in poor mounts of other members of this genus when the heads are twisted a trifle. The appearance is purely an artifact. MacCallum's next point is that this worm has only two parts of the trident present, or that it has, in other words, a bident. The figure does not bear out this assertion; two typical *tridents* are shown, each with three prongs. MacCallum states that the males have six pairs of pre-anal papillæ and three post-anal pairs.

The description of *C. elegans* is quite brief. The author could not determine whether or not the males have one or two spicula, and because the male tail is "not so complete and pretentious as in *C. floridianæ*" he believes that the worms deserve a new name.

In *C. ptychozcondis* the only feature MacCallum noted that seems at all distinctive is the fact that the intestine follows immediately after the muscular esophagus. If this is the case this worm is not a member of the genus *Camallanus.* The anus is on the left of the worm described. If the observation is correct it is the first recorded instance of which I know in the literature on nematodes in which the anus does not lie in the sagittal plane. I am of the opinion that the worm or worms studied were twisted in mounting or handling. Unless a series of sections of this worm can be shown in which the lateral lines are in the same

plane with the anus, I can not accept MacCallum's conclusion. He says "that the vagina has not been seen, but appears to be very near the mouth on the left side." If it were not near the middle of the body and in the mid ventral line I would accept with some caution his conclusion, because this is the characteristic position of the vulvar opening in this genus.

MacCallum's *C. cyathocephalus* is clearly the fourth stage of his *C. scabræ*. I have described in detail this same stage for *C. americanus.*

Unfortunately the description of *C. bungari* is so incomplete and the drawing so obscure that it is impossible to locate the worm even with regard to its genus. Certainly with the slight description given it could not be placed in the genus *Camallanus*, because of the single portion of the esophagus and the type of the oral apparatus. The picture seems to show eggs in the terminal portion of the uterus; if the worm were mature, larvæ only would be found in this portion of the uterus if it belonged to the genus *Camallanus.*

Since I have found *C. americanus* in many different species of turtles in the United States, I have tried to decide whether or not MacCallum found this species and described it as one of his new species. In going over his paper carefully I am unable to admit that from his descriptions and figures he described the worm I recorded under the name *Camallanus americanus*, although it is possible that he might have had the worm. One can not be certain whether or not he had some of the species described by Leidy, nor can one be certain of the validity of any species which he describes.

In the face of these facts it seems to me that at present it would be impossible to accept these species in the genus *Camallanus* and hence I suggest that they be considered as *species inquirendæ*. *Camallanus* is such an important genus that the species described within it should be established without any doubt whatsoever. It is unfortunate that MacCallum did not publish more definite data concerning the forms and it is to be hoped that he will follow up his paper with a complete description of each worm, based on the study of more material and histologic sections.

In the past, nematode descriptions have for the most part been rather superficial and a great deal of confusion has arisen concerning the identification of genera and species. The worms are

so difficult to study on account of the problem of proper technic that it is not surprising that many incomplete descriptions should have been published. It is to be hoped, however, that the day will come when nematodes will be as thoroughly studied and described as other parasitic worms have been and that their classification and identification will be made more certain.

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## AN AMICRONUCLEATE RACE OF PARAMECIUM CAUDATUM

PROBABLY no representative of the Protozoa has received more attention in matters relating to life cycles, reproduction, heredity and cytology than has *Paramecium.* It should be of general interest, therefore, to record the occurrence of a race of *Paramecium caudatum* which appears to be entirely devoid of a micronucleus. The recent studies by Dawson (1) on an amicronucleate race or species of *Oxytricha* add interest to the present discovery.

In the fall of 1914 Doctor M. H. Jacobs of this Laboratory used, in certain heat experiments, some *Paramecium caudatum* derived from a culture which exhibited great viability. During the following January Hance (2), in examining some of the sur-

viving *Paramecia*, found that a few were characterized by the presence of three contractile vacuoles instead of two, the normal number. Several of these animals were isolated and became the progenitors of the multivacuolate race studied by Hance (2). In speaking of the cytology of the race Hance mentions the great difficulty experienced in staining the micronucleus and states that "the depression in the macronucleus where the micronucleus usually lies is frequently visible but it appears quite empty." He decided, however, that there was one micronucleus present.

The greater viability and the slightly larger size of this race as compared with the wild races led to its use for class work. Having occasion, during November, 1919, to filter about four hundred cubic centimeters of classroom culture densely populated by this race of *Paramecium* the writer fixed the animals so obtained in warm Schaudinn's sublimate alcohol and subsequently stained them with Delafield's hæmatoxylin. On examination it was discovered that in none of the individuals could a micronucleus be found. This observation in itself was not conclusive since the seeming absence of the micronucleus might have been due to faulty technique. The same material was stained with borax carmine and the absence of the micronucleus as a staining body was confirmed. Later four different fixatives were used and the material stained with Carmalum and in no case was the micronucleus found. Material was then fixed daily from a series of four cultures for periods ranging from two to four months. Throughout this period the character of the *Paramecia* remained constant in that no multivacuolate animal possessed a micronucleus.

For obtaining pure lines of amicronucleate animals with which to make further observations twenty multivacuolate individuals were isolated. Some of the progeny of each of these were stained in aceto-carmine and in each case the micronucleus was absent. The question as to the identity of the multivacuolate race and the amicronucleate race then arose. The fact that the progeny of twenty multivacuolate individuals showed no micronucleus supported this supposition. A number of slides, made before the discovery of the multivacuolate race by Hance, were found in the Laboratory by Doctor D. H. Wenrich, to whom the writer is greatly indebted for his constant interest, valuable advice, and criticism throughout this preliminary work. Both micronucleate and amicronucleate individuals are to be

found on these slides. Several amicronucleate individuals contain three distended contractile vacuoles. All the micronucleate individuals contain only two. There is reason to believe that these slides were made from the same cultures from which Doctor Jacobs obtained his animals for experimentation. Therefore these slides also indicate the identity of the two races. Apparently both the extra vacuoles and the absence of a micronucleus were characters present before the heat experiments referred to.

Throughout the entire history of the cultures observed the lightly stained, comparatively large, very irregular, and expanded macronucleus is characteristic of the race. Under poor cultural conditions animals with regular nuclei are few in number and these nuclei are usually oval in shape and proportionately larger, more lightly stained than others and often blending with the cytoplasm. Under the same conditions condensations of the chromatin material are of frequent occurrence and consist of three types: (*a*) small or large tongues of chromatin, compact and darkly stained throughout or only around the edges, usually lying in a concavity of the macronucleus, (*b*) small, circular, dense masses of chromatin, usually flattened and near the surface of the macronucleus, (*c*) bar-shaped condensations, many times longer than broad, ranging from very loose aggregations of granules to very compact masses.

In the early part of the work the writer often experienced difficulty in deciding whether or not a micronucleus was present because macronuclear condensations frequently resembled micronuclei. But after observing many specimens they were easily distinguishable since neither condensations, lobes, nor detached portions of the macronucleus possessed the detailed structure typical of a micronucleus. They could always be identified on very careful examination as portions of the macronucleus by the arrangement of the chromatin. It is possible that Hance may have seen macronuclear condensations resembling a micronucleus or small detached portions of the macronucleus which at certain times are rather common.

Other differences between the nuclei of micronucleate and amicronucleate animals were noticed. The macronucleus of the wild, micronucleate races is compact, comparatively small and darkly stained with a distinct concavity for the micronucleus. The nucleus of the amicronucleate race is large, expanded, and lightly staining.

In addition to these characters there are also certain other morphological characters which distinguish the race. The amicronucleate race is larger than the wild ones so far observed. The curve of the buccal groove is slightly greater than that of the micronucleate animals. The posterior tip is slightly bent toward the aboral side and the buccal groove itself is shallower since the sides of the groove are bent outward. All evidences so far indicate that the amicronucleate race has the potentiality of forming from three to seven contractile vacuoles.

Attempts have been made by the writer to induce conjugation. A flourishing culture has been allowed to evaporate to half volume and small mass cultures have been submitted to various experimental conditions but so far the writer has been unable to induce conjugation in this race. Hance (2), however, induced conjugation in the multivacuolate race by the method first mentioned. Amicronucleate animals in conjugation are to be found on the slides (made before Hance's discovery of the extra vacuoles) mentioned above. Hence the race has conjugated in the past and attempts will be made to induce conjugation in the future.

The main question in the future study of this race will be the cytology of the conjugation process. This will require experimental work on methods of inducing conjugation in the race and the study of the conjugants so obtained. The effect, if any, of the absence of the micronucleus on the division process will also be observed.

The maintenance of pure lines and the study of the nuclear changes which proceed in ordinary vegetative existence will also be an important part of the future work. If there is a process of endomixis the same means will provide a basis for the study of that phase.

These two matters are the most interesting from the standpoint of cytology, especially since the work of Calkins and Cull (3) on conjugation, and Erdmann and Woodruff (4) on endomixis, in *Paramecium caudatum* show that the active body is the micronucleus and that the macronucleus breaks down and disappears in both processes.

Is the macronucleus affected by different cultural conditions in any definite way and how does the behavior of the nuclei of the micronucleate race compare with the behavior of the macronucleus in the newly discovered race? The preliminary work done so far indicates that there is a definite relation between

environment and the behavior of the macronucleus and that the macronucleus assumes different shapes and appearances under different cultural conditions.

Is this amicronucleate paramecium able to exist indefinitely without conjugation involving a micronucleus and without reorganization of nuclear material, or is there another type of reorganization in this race? A nuclear reorganization, if present, must evidently be of a different type from that described by Erdmann and Woodruff.

These and similar problems are interesting, not only in themselves, but because *Paramecium* has been studied in great detail by Jennings and others with reference to the occurrence of cytoplasmic variations. The amicronucleate race, however, is important because the variation is one of nuclear structure. The importance and interest of the study is increased by the fact that the micronucleus is usually considered to be an aggregation of generative or hereditary chromatin and the body which supposedly initiates reproductive processes of all types and from which, in sexual reproduction, the new nuclei are formed.

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## NOTE ON THE OCCURRENCE OF A PROBABLE SEX-LINKED LETHAL FACTOR IN MAMMALS

THE occurrence of sex-linked lethal factors in *Drosophila* is a matter of common knowledge to most biologists. Since mammals have an essentially similar type of sex determination in so far as their dimorphism of sperm is concerned, it is theoretically possible that sex-linked lethal factors should occur among them.

Inasmuch as only a few sex-linked factors of any sort are known in laboratory mammals where numerous other genetic differences have been recorded, breeding tests of linkage relations by ordinary methods are precluded. The first indications of a sex-linked lethal factor would therefore be expected to consist of an abnormal sex ratio at birth and a reduction in the size of litters.

Such conditions are met with in certain Japanese waltzing mice derived from a remarkably closely inbred race. This particular strain of Japanese mice has been inbred from the descendants of a single pair of animals for approximately fourteen years. The animals are difficult to raise and the litters are frequently distinctly smaller than those of non-waltzing mice of inbred races. The great majority of animals of this strain have been bred by Mr. George Lambert of Boston, who has provided adult or young adult animals to the writer for several years. Mr. Lambert is now engaged in recording sex ratio data at the birth of litters and states that at present an excess of females is being produced in his stock. While awaiting increased numerical data from his records, however, it appeared advisable to make a short note of the sex ratio and behavior of this strain in the animals raised under controlled observation.[1] The sex ratio of inbred non-waltzing mice is $103.1 \pm 2.8$, showing a slight excess of males. The sex ratio obtained from Japanese waltzing race of the Lambert strain is $53.2 \pm 5.7$. The difference between these ratios is 7.9 times its probable error and is certainly significant.

Since recessive sex-linked lethal factors are transmissible through a female to one half of her male offspring, the sex ratio among the progeny of females carrying sex-linked lethal factors should be theoretically 50.0. Males of the Japanese waltzing race can not, of course, transmit the lethal factor, since if they possessed the factor it would result in their death. If then animals of the Japanese waltzing race are crossed with non-waltzing mice of a race presumably free from lethals, the reciprocal crosses should give markedly different sex ratios. Such is actually the case. Non-waltzing females by Japanese waltzing males should give a sex ratio free from the effect of lethal factors. The observed sex ratio in the $F_1$ generation of this cross is 118.2

[1] Dr. H. J. Bagg, of the Memorial Hospital, New York City, has kindly placed at my disposal data collected by him while breeding Japanese waltzing mice of the same (Lambert) strain. These data are included with my own in this publication.

$\pm$ 3.8 and in the $F_2$ generation 113.3 $\pm$ 3.0. The combined ratios are 115.9 $\pm$ 2.7. The significant excess of males above that found in the inbred non-waltzing stock is a common result of hybridization. The reciprocal cross gives a most interesting contrast. When Japanese waltzing females are crossed with non-waltzing males the sex ratio is 44.0 $\pm$ 7.4. This differs from the sex ratio of the reciprocal cross by 8.9 times the probable error of the difference.

Certain other evidence is obtainable from the results of mating Japanese waltzing females or their female descendants with hybrids of the $F_1$ or back cross generation. Such crosses give opportunity for a lethal factor to be transmitted and to express itself. We should expect, therefore, that an excess of females would be obtained in the progeny of such crosses. Such is actually the case, the ratio being 78.7 $\pm$ 2.9. The exact ratio to be expected would depend upon the numerical relation of young in a given population, obtained from homozygous normal females, to those from females carrying the lethal factor.

The size of litters given by Japanese females irrespective of the sire is distinctly smaller than that of litters from non-waltzing females. The average of 58 litters from Japanese waltzing females is 3.38 and from non-waltzing females (100 litters) 5.93. The litters from Japanese females are therefore .57 times the size of those from non-waltzing females. This result is in general accordance with the presence of a sex-linked lethal factor. $x^2$ test of the frequency distributions of litters in the two cases shows that the odds are less than one in 1,000,000 that they are the same.

While it is realized that the above evidence is preliminary, its entire consistency and the controlled nature of the material makes it seem likely that *the observed figures indicate the presence of what is apparently the first case of sex-linkage in rodents and of a sex-linked lethal factor in mammals.* The possible exceptions to this statement[2] are to be found in the case of hemophilia recently reviewed by Whitman[3] and in the possible difference in reciprocal crosses between *Epimys rattus* and *Epimys alexandrinus* reported by De l'Isle and reviewed by de Meijere.[4]

[2] Progressive muscular atrophy in man has a lethal action. Its action is, however, so delayed that it seems scarcely to fall into the same category with what have hitherto been considered as sex-linked lethals.

[3] *Jour. Cancer Research,* 1919, 4, 181.

[4] *Archiv. f. Rassengesell. u. Biol.,* 8, 697.

In order to explain the case reported by Whitman an entirely new and unsupported behavior of the lethal factor is hypothesized. This consists in a supposition that females possessing two does of the lethal die. Inasmuch as a female with two doses of a sex-linked lethal could not be formed except by mutation, provided the relations observed in *Drosophila* hold true, the presence of a sex-linked lethal factor of the accepted type does not appear to be strongly supported. In the case of the rats a simple statement of a difference in reciprocal crosses is the sole evidence. In this case no lethal action is apparently involved; but sex-linkage might possibly account for the result. Until actual experimental evidence on this matter is available, however, it seems as though it was not sufficiently definite to be considered as having previously established the existence of sex-linkage in rodents.

C. C. Little

## CONCERNING THE FOSSILIZATION OF BLOOD CORPUSCLES

Recently, while studying a series of microscopic preparations of fossil material in connection with paleopathology, I observed in sections of a dinosaur bone (possibly *Apatosaurus*) which I had collected in the Como beds of Wyoming in 1906, some ovoid bodies, arranged around the periphery of vascular spaces and Haversian canals, which looked remarkably like blood corpuscles. Close scrutiny of the available material, however, did not satisfy me that the objects might not be the products or by-products of incomplete crystallization. The majority of the bodies had the size and shape of modern reptilian erythrocytes; the nucleus of course not being evident, since only the outward form of the corpuscle was to be seen. Other bodies, apparently similar, were irregular in shape and hard to distinguish structurally from the regular bodies. These latter, however, may be masses composed of several corpuscles which had become agglutinated.

Not being satisfied with the results of my observations, I should not have published anything about it had I not seen in a memoir by Seitz[1] a description of similar bodies in sections of normal

[1] Adolf Leo Ludwig Seitz, 1907, "Vergleichenden Studien über den mikroskopischen Knochenbau fossiler und rezenter Reptilien und dessen Bedeutung für das Wachstum und Umbildung des Knochengewebes im allgemeinen," *Nova Acta. Abh. der Kaiserl. Leop.-Carol. Deutschen Akad. der Naturforscher. Halle,* Bd. LXXXVII, No. 2, 329–330, Tab. XXI, Fig. 61, where the corpuscles are shown in a photomicrograph in 365 diameters.

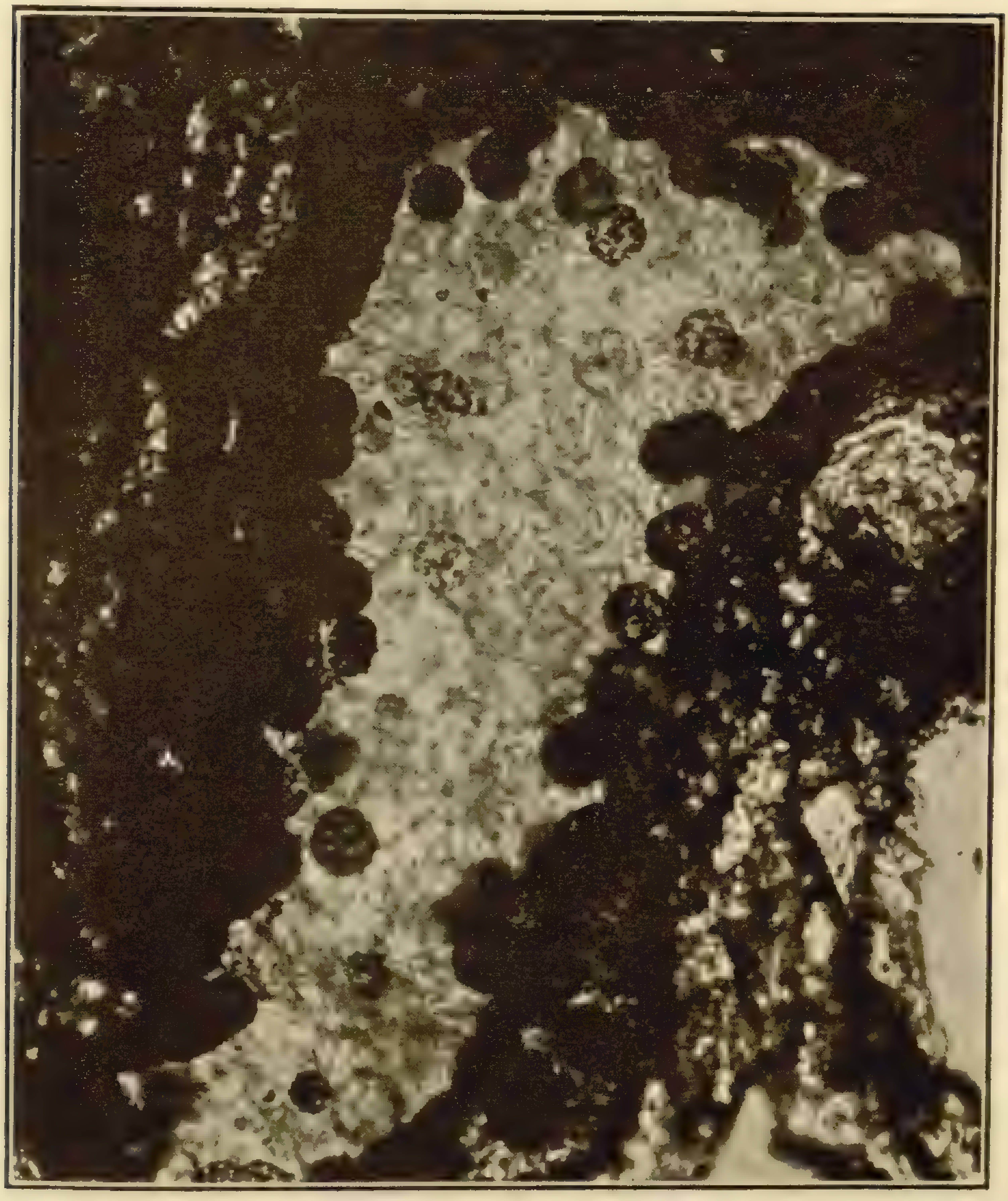

FIG. 1. A vascular space in a normal metatarsal of *Apatosaurus,* or some related dinosaur, from the Como Beds of Wyoming, showing in the rounded marginal bodies, the preservation of supposed blood corpuscles. Magnified 200 diameters. These are the same bodies that Seitz saw in European dinosaurs. The light area is the vascular space filled with clear quartz. The dark marginal areas are osseous trabeculæ rendered dark by iron. The sharp indentations in the border of the vascular space are interpreted by Seitz as Howship's lacunæ, in which case the rounded bodies would be osteoclasts and not blood corpuscles. Renault searched for, but failed to find, blood corpuscles in bone from the Permian of the Autun basin of France.

bone from the European dinosaur, *Iguanodon Bernissaertensis* from the Wealden of Bernissaert, Belgium. Seitz's description of the blood corpuscles follows:

A larger part of the Haversian canals of *Iguanodon* is empty. A part of them, however, contain small, round, biconvex bodies, apparently with flat surfaces, which occur regularly or scattered about in the lumen of the vessels, with an occasional one near the periphery. Not seldom a compact mass of them entirely fills the blood-vessel. Professor Solereder of Erlangen declares that the bodies are not of plant origin

(spores), and by polarization it is determined that the bodies resemble somewhat crystalline concretions, so that we are forced to the conclusion that we have here some fossilized blood corpuscles. The partial filling of the blood vessel may be due to coagulation or a peripheral thrombus. There is also to be found frequent accumulations of reddish crystals which resemble hæmatoid crystals, and which support the suggestion as to the nature of the material. I give these observations with some reservation.

We may gain an insight into the possibility of the fossilization of blood corpuscles by studying the results of the researches into the nature of the mummified brain material of the ancient Egyptians. This subject has been studied by Mair,[2] who finds that the lipoids of the brain from Coptic bodies, 500 B.C., had been changed into cholesteryl stearate and palmitate.[3] Mair obtained cholesteryl stearate by heating cholesterol with stearic acid, and one may infer that the heat of the desert sands in which the bodies were buried may have been an important factor in the conversion of the brain lipoids into the two relatively resistant substances, palmitate and cholesteryl stearate. These brains, even those dating from a period prior to the process of embalming (4500 B.C.), are frequently so well preserved, though greatly shrunken, that practically all the gyri may be accurately determined. This item from more recent times may aid in an explanation of processes occurring in geological ages.

The studies on Egyptian mummies have not resulted in the discovery of blood corpuscles. Schmidt[4] examined bodies dating from 1000 years before Menes (3400 B.C.) to 500 B.C. (mummified material from Coptic bodies) and was unable to find a positive hæmin reaction, tending to show the complete disappearance of all blood in the process of time. Wood Jones,[5] however, is convinced that traces of blood are readily discernible. Elliot Smith has referred to blood stains on bandages used in

[2] W. Mair, 1913, "On the Lipoids of Ancient Egyptian Brains," *J. Path. and Bacteriol.*, XVIII, 179–184; 188.

[3] Mair's results are confirmed and extended by Lapworth and Royle, 1914, "The Lipoids of Ancient Egyptian Brains and the Nature of Cholesteryl Esters, *J. Path and Bacteriol.*, XIX, 474–477.

[4] W. A. Schmidt, 1907, "Chemische und biologische Untersuchungen von ägyptischen Mumienmaterial, nebst Betrachtungen über das Einbalsamierungsverfahren der alten Aegypter," *Ztschr. f. allgem. Physiol.*, VII, 369–392.

[5] F. Wood Jones, 1908, "The Post-mortem Staining of Bone Produced by the Ante-mortem Shedding of Blood," *Brit. Med. J.*, 1, 734–736.

the primitive surgery of Egypt. Ruffer in his extensive studies into the histology of Egyptian mummies did not discover any definite corpuscles.

It may be of interest to note that Friedenthal[6] announced to the physiological society of Berlin the discovery of red blood in the body of a mammoth from eastern Siberia which had been frozen in the tundra since Pleistocene times. The precipitin reaction of the blood is similar to that of the modern elephant. No record is made of the preservation of blood corpuscles. While this is an extremely interesting discovery, it must be recalled that cold brings many chemical reactions to a halt, and there may have been little change in the blood of this mammoth during its 175,000 years of cold storage in the Siberian mud. The body had been so well frozen that the flesh was still fresh enough to satisfy the hunger of wolves and dogs.

Hoppe-Seyler has shown that dried red blood corpuscles of man contain 2.5 parts of cholesterin in 1000. While this is an extremely small amount of lipoid substance, since it is chiefly in the cortex of the corpuscle, it occurred to me that this might offer an explanation of the preservation of blood corpuscles. That is, under favorable conditions, the lipoids of the blood might be changed into some resistant substance like palmitate or cholesteryl stearate and thus retain the form of the corpuscles and delay their destruction long enough for fossilization to set in; these substances being replaced later by the mineral crystals from the magma in which the body was immersed. The beautiful little ganoid fish brains described by the writer[7] some years ago from the Coal Measures may have been preserved in a similar way. The resemblance between brain substance and blood corpuscles is close in this respect that each has a small amount of resistant substance, a large amount of water and a relatively similar proportion of lipoids which may have become transformed, under proper conditions, into resistant substances which carried the part over the critical period of destruction.

In view of the fact that so many soft-bodied animals are so beautifully preserved in the rocks, that the histological nature of Paleozoic muscle tissue has been determined, that bacteria and the delicate parts of flowers are so frequently fossilized, it

[6] *Deutsche Med. Wochenschrift*, 1904, p. 901.

[7] Roy L. Moodie, 1915, "A new Fish Brain from the Coal Measures of Kansas, with a Review of other fossil Brains, *J. Comp. Neurol.*, XXV, No. 2, 135, 17 figs.

is certainly not beyond reason to expect the preservation of blood corpuscles. The subject is still an open one but this contribution to the theory of fossilization, it is hoped, may help to clear up the matter of the preservation of delicate objects.

The fossilization of any of the blood crystals as suggested by Seitz[1] is extremely improbable, since the evanescent nature of the crystals of hæmoglobin is well known. Whether the crystals seen with the supposed blood corpuscles have resulted secondarily from the disintegration of hæmin crystals or whether the whole appearance is due to chemical reactions in the incomplete crystallization of inorganic substances is an open question. Still we must not close our eyes to the possibility of discovery and block the way to progress by saying it is either one or the other. This paper merely opens the field.

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# TYPES OF WHITE SPOTTING IN MICE[1]

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THE occurrence of white spotting in the coats of colored mammals is one of the commonest phenomena encountered by the student of variation and heredity. For a long time spotting was thought to be of the same nature as albinism, a condition in which no pigment is present in the fur and eyes, leaving the pelage clear white and the eyes pink. Many specimens of white spotted animals are still to be seen in museums masquerading as "partial albinos."

As soon as the methods of experimental breeding were employed in studying such variations, albinism and white spotting were found to be genetically distinct. Albinism, because of the striking nature of the variation and its almost identical appearance wherever encountered was one of the first mammalian variations to be analyzed and its mode of inheritance is now well known. The case of white spotting is quite different. The spotting of most animals presents an extremely wide range of variation. An almost continuous series may be traced by the casual observer from the extreme spotting of white dogs with black eyes to the small star or blaze on the foreheads of some colored horses. The solid colored condition, generally known as self, and spotting may thus in some instances be distinguished only by the presence of a few white hairs. Moreover, the inheritance of white spotting in different animals and of the various grades of

[1] The experiments reported in this paper were performed at the Bussey Institution, Harvard University, Forest Hills, Mass.

spotting in the same animal has been found by experiment to be distinctly different. Our knowledge of the hereditary factors and of the processes concerned with the development of pigment in the coat is still too fragmentary for a satisfactory comparative exposition of the nature and causes of white spotting. The need at present appears rather to be for intensive experimental studies of variations in the most favorable species in which they occur. The white spotting of some species also provides excellent material for the study of the nature of genes with small quantitative effects either as main or as modifying factors. As a contribution to these ends the present report of experiments with white spotting in mice is offered.

In the house mouse the whole range of variability in white spotting is found. At one extreme are black-eyed whites, with pigment occurring only in the eyes; at the other extremes are colored mice which have only a few white hairs on forehead, feet, tail or belly. The appearance of all possible intergrades between black-eyed whites at one end of the scale and animals closely resembling self at the other led Cuenot to suppose that all spotted mice differed from self by a single main spotting factor (*P*) which might be present in various conditions represented by factors with minor effects which caused the more apparent differences in amount of pigmented areas. The finer details and intergrades of spotting he regarded as purely somatic variations with no germinal (hereditary) cause. Later, however, Little (1915) bred spotted mice and classified all parents and offspring by estimating the percentage relations between white and colored spaces. He found that the "continuous" series of spotted forms consisted of two main types. One of these, black-eyed white, was characterized by a pelage practically all white with dark eyes. The other, piebald, was distinguished by the greater extent of pigmented areas down to and including mice with only a few white hairs dorsally and a small patch of white on the belly. Another type of spotted mouse known as "blaze" and

characterized only by a small white spot between the eyes was also removed from the continuous series by the work of Little (1917). This variation was apparently heritable, although subect to some variability in expression. Two types of piebald spotting, one with more and one with less white were likewise indicated in Little's data. He regarded these differences as possibly due to two distinct modifying factors of the piebald gene. In addition, crosses of black-eyed white with self-colored mice had produced two new spotted types, one with more and one with less white. The continuous series of spotted forms has thus been broken up on the basis of amount and distribution of spotting into a large number of fairly distinct types, two of them due to genes the inheritance of which is known. The process of resolution has not reached its end yet, for a great deal of variability exists within the various types, and the resolution of these variations into still more sub-types is possible. The specific objects of this study are to redefine the ranges of the main types of spotting in the light of increased data; (2) to find out, if possible, whether the conditions of spotting in the sub-types are due to different combinations of the main genes or to distinct genes which modify the expression of the main genes.

The two main types of spotting in mice are known respectively as black-eyed white and piebald. Little at first described black-eyed whites as spotted mice which were 95 per cent. or more white dorsally, later extending this limit to include mice which are 80 per cent. or more white dorsally. Certain yellow black-eyed whites were described by Little (1917) as exhibiting as little as 60 per cent. of white in the dorsal surface. Evidence will be presented later to show that the range of black-eyed white variability is even greater than this and may include mice with as little as 50 per cent. of dorsal white.

Piebalds are much darker, *i.e.,* have less white spotting than black-eyed whites. The piebalds born in my experiments have, with one or two exceptions, been less than 50 per cent. white dorsally with belly spotting rang-

ing from 12 per cent. to 85 per cent. of white. Other investigators have recorded piebalds whiter than this[2] and one or two of my crosses indicate that piebalds may exhibit as much as 60 per cent. or 65 per cent. white, although such animals have been extremely rare in these experiments.

Genetically these two types of spotting are distinct, and each is due to a gene distinct from and independent of the other. When black-eyed whites are crossed with piebalds equal numbers of black-eyed whites and piebalds result. When black-eyed whites are bred *inter se*, black-eyed whites and piebalds result in the ratio of 2:1. The black-eyed white condition is therefore due to a gene (symbol *W*) acting with the gene for piebald (symbol *s*). Black-eyed whites are heterozygous for the gene *W* and are homozygous for *s*. Genetically they are *Wwss*. Piebalds may be represented by the formula *wwss*, where *w* stands merely for "not-black-eyed white." These two genes, as is known from previous data (Little, 1915; Dunn, 1920), are neither allelomorphic nor linked, but entirely independent.

As additional evidence of the distinctness of these types and to illustrate the comparative ranges as regards amount of white spotting on the dorsal surface, the data from a number of crosses between black-eyed whites and piebalds are presented in Table II, Cross 1, and Fig. 1 solid line. All mice in this as in other distributions to be discussed were graded by estimating the amount of white in the coat. The two surfaces, dorsal and ventral, were graded separately, the total of each surface being regarded as 100. The percentage of dorsal white was expressed as the numerator and the percentage of ventral white as the denominator of a fraction. Thus a mouse entirely white dorsally and ventrally (except for pigment in the eyes) was graded as 100/100; while one with only a small patch of white ventrally was

[2] For instance, the race of pure Japanese piebalds described by Little (1917) which varied from 100 per cent. to 64 per cent. white dorsally and closely resembled black-eyed whites in amount of spotting.

graded 0/1, or 0/3, etc. The results can be easily transposed into Little's scale by simple subtraction of any grade from 100, since his percentage expressed the amount of color. For convenience, only the dorsal grades have been used in the tabulation, since the results

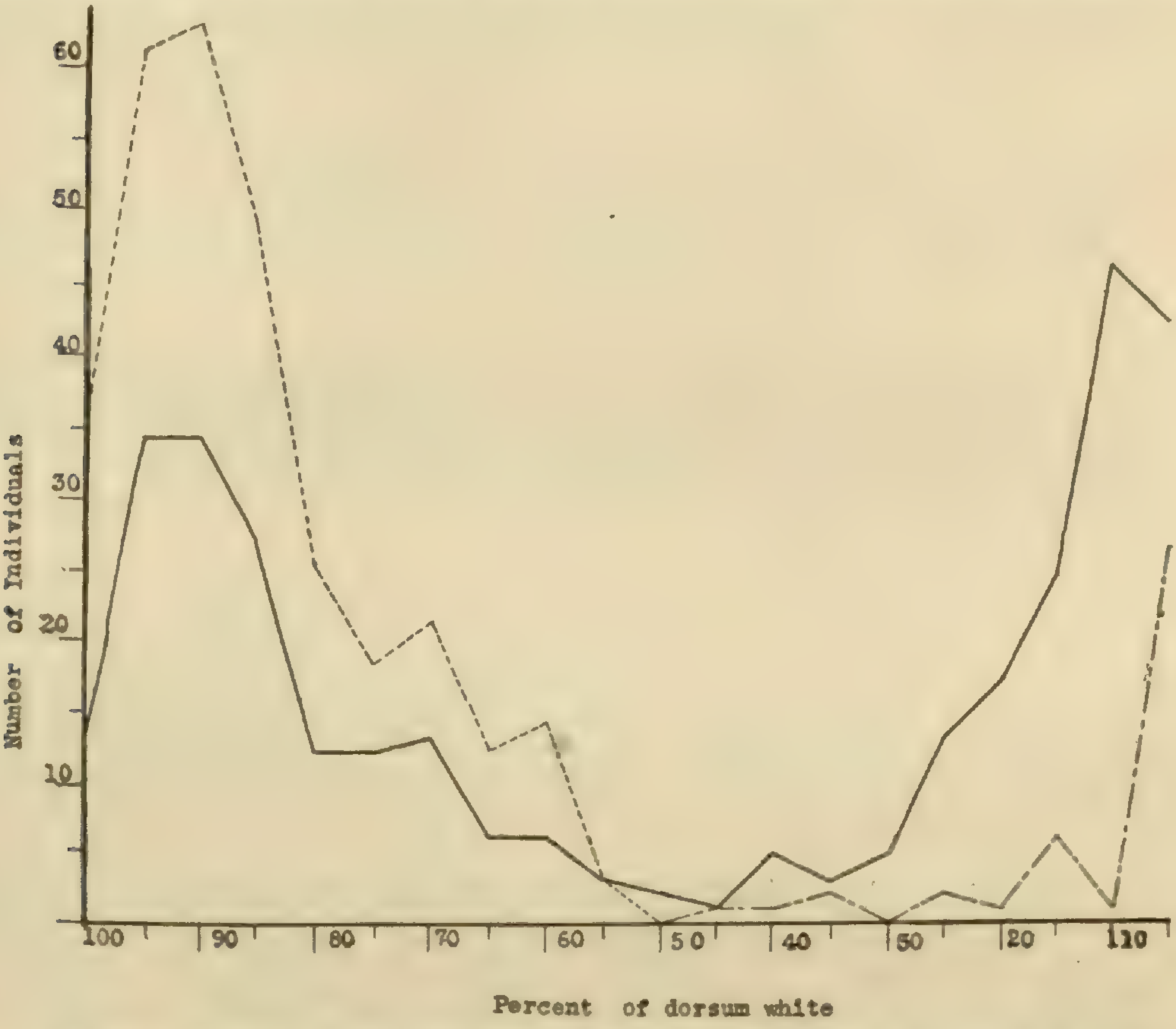


FIG. 1. Showing graphically the distribution as regards amount of white spotting of (1) all black-eyed white mice (dotted line); (2) offspring of crosses of black-eyed white by piebald (solid line); (3) offspring of cross of black-eyed white by self (broken line).

obtained by using the ventral grades are in all respects similar.

Of 323 mice raised from crosses of black-eyed whites by piebalds, approximately half (160) were 50 per cent. white or more; the other half (163) were spotted mice with less than 50 per cent. white; expected 161.5 of each. No mice were obtained early in the experiment which were from 55 to 51 per cent. white and it was thought that this zero class indicated a clear discontinuity between the types. With larger numbers, however, three mice of this sort were born. That the division between black-eyed white and piebald at 51 per cent. is, however,

not an arbitrary one is shown by the bimodal nature of the curve (Fig. 1, solid line), which divides itself near 50 per cent.; by the closeness with which the actual assortment of *W* and *w* (assuming the lower limit of *W* spotting as 51 per cent.) approaches the expected and by breeding tests of spotted mice slightly more and slightly less than 50 per cent. white. Those more than 50 per cent. white proved to be black-eyed white and those less than 50 per cent. white proved to be piebalds.

The mode of the black-eyed whites in this distribution is at 95–86 per cent. white, although when all other black-eyed whites are added (Table II, Cross 8, Fig. 1, broken line) the main mode is found to be at 90–86. The range is from 100 per cent. to 51 per cent. and the mean of all black-eyed whites is 83.5 per cent. ± .4 with a standard deviation of 11.4 per cent. ± .3.

This establishes a wider range of variability for the black-eyed white variation than has been current heretofore, by the addition to the distribution of the classes less than 80 per cent. white. It will be shown later in the section on modifying factors that these darker classes are not merely somatic fluctuations in the expression of the gene for black-eyed white (*W*) but represent genetic variations. They may be regarded as sub-types of black-eyed white.

### Piebald

The piebalds produced by the cross of black-eyed white with piebald are represented by that part of the solid line (Fig. 1) lying between 50 per cent. and 0 per cent. Unlike black-eyed whites, piebalds are not always distinguishable on the basis of dorsal white alone, for some piebalds have no white at all dorsally. In such cases the ventral white must be used as a criterion, and of the pure piebalds produced in this cross none had less than 12 per cent. of ventral white and this was used as the limit of piebald variability. All animals with 12 per cent. or more of ventral white and with from 0 to 5 per cent. of dorsal white were placed in the 0–5 per cent. class. The correctness of this classification can be inferred from the

closeness with which the numbers of piebalds resulting from various crosses (Table II) agree with the numbers expected on the assumption that the gene *s* segregates at random with regard to *S*, *W*, and *w*. It is certain,

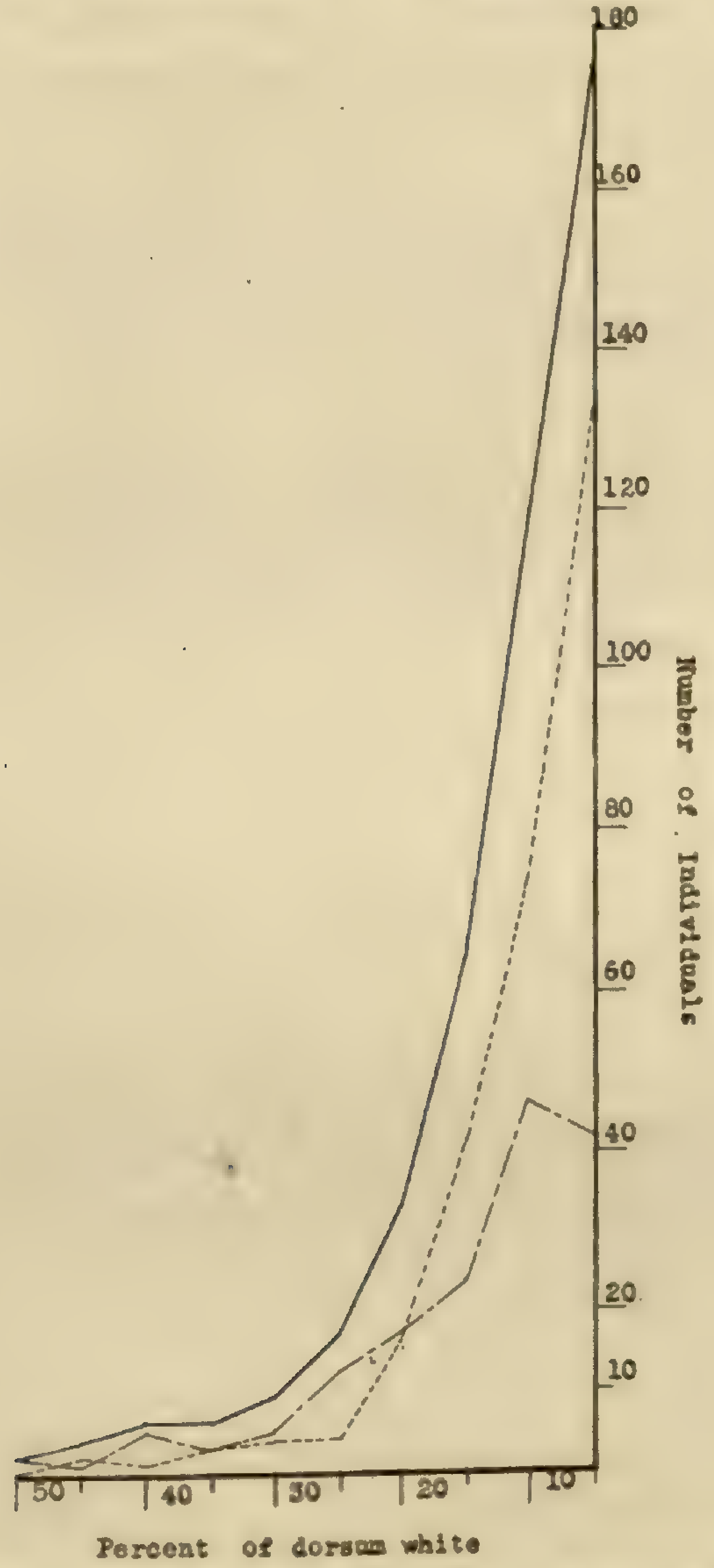


FIG. 2. Showing graphically the distribution as regards amount of white spotting of (1) all piebald mice (solid line); (2) piebald mice resulting in the second generation from a cross of piebald by self (dotted line); (3) piebalds extracted from black-eyed whites (broken line).

nevertheless, that all possible variations of piebald have not been met with in the distributions presented in Table II and Fig. 2. Little (1917) bred a race of pure piebalds which varied from 100 per cent. to 64 per cent. white dorsally. These piebalds are entirely outside the range of the piebalds bred in the present experiments. Moreover,

since the conclusion of these experiments I have been able to isolate one family of piebalds characterized by from 0–10 per cent. of white dorsally and from 0–12 per cent. of white ventrally. They have even produced a small proportion of solid colored young which probably represents an extreme condition of piebald in connection with other subsidiary factors for the increase of pigmented areas. Thus aside from the piebald mice observed in these experiments it can be seen that the genotype "*ss*" (piebald) may vary somatically from solid colored to all white with dark eyes.

As regards only the piebalds observed in this investigation (and this includes all the piebalds in the experiment, 437 in number) it can be seen by a glance at Table II, Cross 11, and Fig. 2 that they constitute a well-defined class ranging from 50 per cent. to 0 per cent. white dorsally. Of the total (437) 295, or 67 per cent., are less than 10 per cent. white. They are less variable than the black-eyed whites, since the standard deviation of 302 black-eyed whites is $11.4 \pm .3$, while the standard deviation of 437 piebalds is $8.6 \pm .2$. This difference is not due to the range which is 50 per cent. in each case, but to the grouping of the piebalds in the two darkest classes while the black-eyed whites are distributed more evenly throughout the distribution. The mean and standard deviation of piebalds out of crosses with self (Table II, Cross 10) are significantly lower than the same constants from piebalds out of crosses with black-eyed white. An interpretation of these differences is offered below in the section on modifying factors.

The main difference between the black-eyed white and piebald condition is due to the presence in the black-eyed whites of a dominant gene (*W*) which the piebalds lack. This gene in single dose, and in connection with the gene for piebald, limits the formation of pigment to, on the average, about 10 per cent. of the dorsal surface, though mice possessing this combination of genes may vary from all white with dark eyes to about 50 per cent. white.

The piebald gene in double dose and acting alone per-

mits the formation of pigment on the average, in about 85 per cent of the dorsal surface, although such mice may vary from solid colored dorsally (0 per cent. white) to 100 per cent. white dorsally.

There are certain other combinations of these genes possible, and by experiment these other combinations have been found to produce other spotted types. Some of these types have been found to be indistinguishable somatically from piebald, although differing from piebald in genetic constitution. The experimental evidence bearing on the appearance, constitution and variability of such additional types follows.

## Type "A" Spotting

When black-eyed white is crossed with self approximately half of the progeny are spotted and half are self or exhibit at most only a small white ventral spot. From such a cross in the present experiments 76 young have been born, of which 40 were spotted and 36 were self colored (Table II, Cross 2). Little found that the self (or nearly self) young from such a cross were ordinary heterozygotes between self and piebald. The spotted young he found to be due to unions of gametes carrying black-eyed white and piebald (*Ws*) with self gametes (*wS*) producing the double heterozygote *WwSs*. He called such spotted mice Type "A". The somatic expression of this combination of genes has produced spotted mice varying from 0 to 45 per cent. of white dorsally (Fig. 1, broken line, and Fig. 3, dotted line). The distribution as regards amount of white dorsally is seen from this figure to resemble closely the distribution of piebald in range, while the mode of Type "A" is at 5–0 per cent. and the mode of all piebalds is at the same point. The mean of all Type "A"'s raised (83 in number) was 7.9 ± .6 per cent. white dorsally with a standard deviation of 8.4 ± .4. This is very close to the mean of all piebalds (9.7 per cent. white) and almost identical with the mean of piebalds extracted from crosses of piebalds with self (8.05 ± .3). Moreover, Type "A"'s which have

no dorsal white may be characterized by an extremely small amount of ventral white. Ordinarily mice with less than 12 per cent. of ventral white may be classed as self. I mated together two such apparently self animals out of a cross of Type "A" by piebald. Each had a small white spot on the belly only covering 4 per cent. of that surface in one of the mice and 5 per cent. in the other. They produced two litters, each of which contained two black-eyed white mice. They were Type "A" mice and not selfs as they had been recorded. A similar case has been reported by Little (1917) in which eleven yellow mice with 6 per cent. and more of ventral spotting proved on breeding tests to be Type "A". That an extreme of Type "A" spotting approached the self condition so closely was not discovered until most of the animals had been graded. It is possible, therefore, that where Type "A" and self animals appear in the same distribution, the self class may be factitiously enlarged at the expense of the Type "A" class, due to errors in grading. Type "A" spotted mice are hence indistinguishable somatically from piebalds, and in certain cases, from selfs, although possessing a genetic constitution entirely different from either of these latter forms.

## TYPE "C"

There remains one other type of spotting to be discussed which like Type "A" and piebald has a different genetic constitution, but which is difficult to distinguish from either Type "A" or piebald. Such mice are produced when Type "A" animals are interbred. Each Type "A" produces gametes *WS*, *Ws*, *wS* and *ws* which by random union give the following array of zygotes:

4 pure for *W* which are non-viable and die in utero.
4 *WwSs*—Type "A"
2 *wwSs* } self
1 *wwSS* } self
2 *Wwss*—black-eyed white
2 *WwSS*—dark spotted (Type "C")

$\frac{1}{16}$ *wwss*—piebald

The visible distribution should consist of approximately 2 black-eyed whites, 7 spotted, and 3 self. The experimental numbers for this cross (Table I, Cross 4) in the present study are 16 black-eyed whites, 27 "spotted,"[3] and 18 selfs. Among the spotted forms is included a new genotypic class consisting of mice which are heterozygous for *W* but pure for self. Little called this genotype "dark spotted," but since somatically it is no darker than other spotted types I shall refer to it arbitrarily as Type "C." Some of the spotted mice from the above cross (4) were tested by crossing them with piebalds. If the mouse tested were piebald, it should produce only spotted (piebald) mice; if it were Type "A" it should produce black-eyed whites, spotted and selfs; while if it were Type "C" it should produce only spotted (Type "A") and self. Out of a number of mice so tested only seven proved to be Type "C" and the only evidence available on the appearance of Type "C" mice is from the appearance of these tested animals. Five were less than 5 per cent. white dorsally; one was 7 per cent. white and one was 8 per cent. The white spotting in all of them was confined to the head, either as a spot on the nose or between the eyes ("blaze"). It is to be regretted that no animals from this cross with less than 12 per cent. of ventral white were tested, since it seems probable from the excess of selfs recorded that some mice graded as self and were really Type "C." The same excess of selfs was noted in a larger amount of data on this cross reported by Little. Its significance is probably the same, that is, it is due to the production of an extremely small amount of spotting by the genetic Types "A" and "C."

Crosses of Type "C" with piebald produced a total of 85 offspring (Table II, Cross 6), of which 43 were spotted and 42 were self (expected 42.5 of each). The spotted

[3] Under the general term "spotted" are included all those genotypes which are somatically indistinguishable when occurring in the same distribution, viz., Type "A," Type "C," and piebald.

mice were genetically Type "A" (*WwSs*) and their distribution is shown graphically in Fig. 3, broken line. This distribution resembles that of the Type "A"'s produced by crossing black-eyed white with self, except that the former are somewhat less variable, due to the ab-

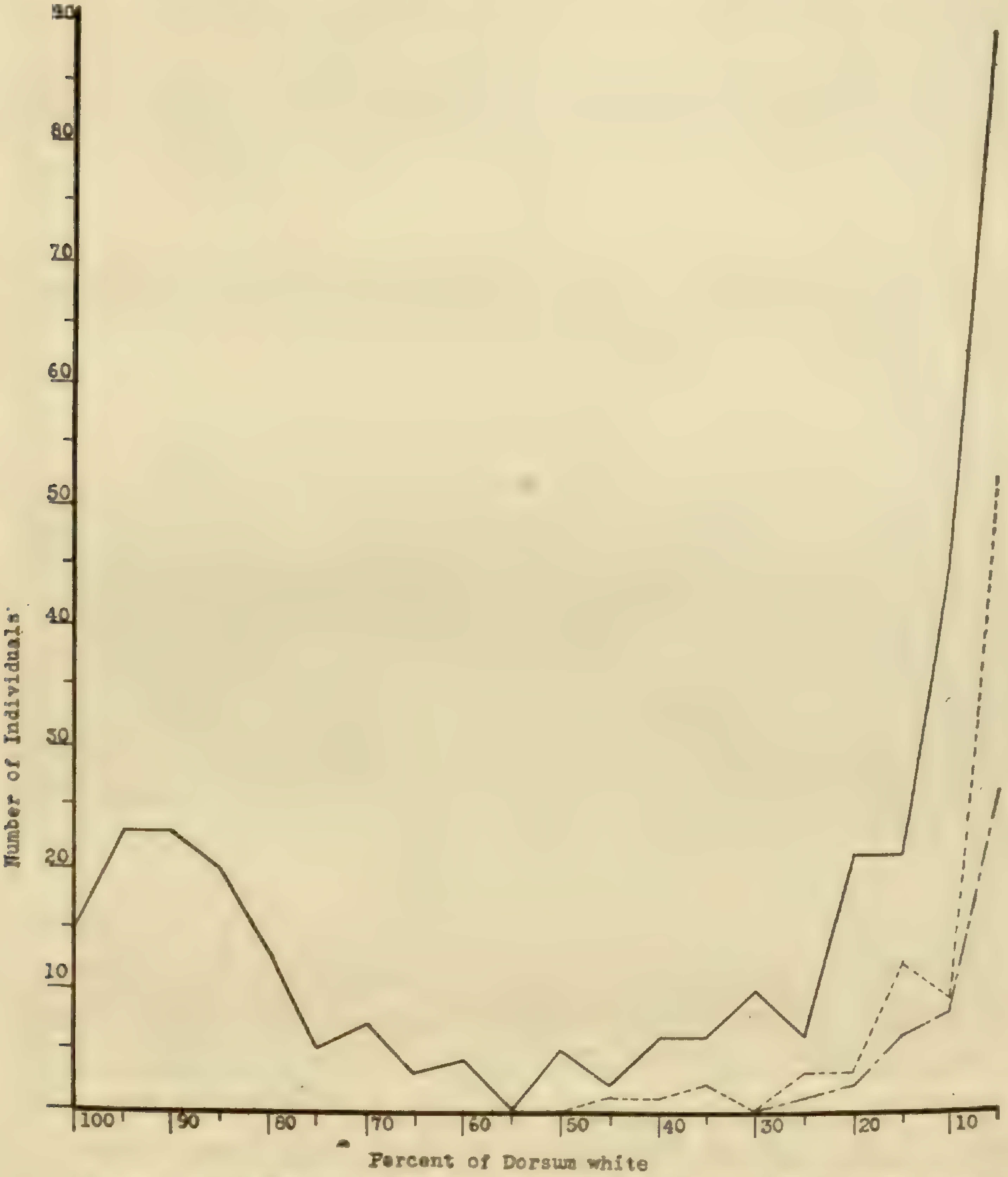


FIG. 3. Showing graphically the distribution as regards amount of white spotting of (1) offspring from crosses of Type A by piebald (solid line); (2) of all Type A mice (dotted line); (3) offspring of crosses of Type C by piebald (broken line).

sence of classes lighter than 25 per cent. white, and their mean is somewhat lower for the same reason. These differences are probably due to the selection of dark Type "C"'s as parents and it will be seen later that the darker

spotted types probably carry modifying genes which have a pronounced effect in increasing the amount of pigment present.

Many crosses were made between Type "A" and piebalds with the object of determining whether the genes *W* and *s* were linked or independent. All offspring from this cross were also graded for variation in amount of white spotting. The distribution of the 443 offspring (Table II, Cross, and Fig. 3 solid line) indicates the correctness of the ranges already established for three types of spotting, since black-eyed whites, "spotted" (Type "A" and piebald) and selfs resulted in the expected ratio of 1:2:1. The range of the black-eyed whites was from 100 to 56 per cent. white; and "spotted" from 50 to 0 per cent. white. The mean of the black-eyed whites was 84.7 per cent. ± .6 of white dorsally with a standard deviation of 10.3 ± .5, which values correspond closely to those calculated for the cross black-eyed white × piebald (Table II, Cross 1). The mean of the mixed spotted was at 11.6 per cent. ± .5 compared with 12.5 per cent. ± .6, the mean of the piebalds in Cross 1. It may be inferred from this that the residence in the same Type "A" zygote of the genes *W* and self has had *per se* no darkening effect on the black-eyed whites and spotted subsequently extracted.

## Self

Data on the ranges of variability of white spotted mice would not be complete without some reference to the variability exhibited by mice which by all tests which have been applied to them are genetically self mice, *i.e.*, lacking the genes at present known to cause white spotting. The heterozygote between self and piebald is generally regarded as self, that is, self is supposed to be completely dominant to piebald. But in a number of cases in these experiments heterozygotes between self and piebald have exhibited a small spot of white on the belly, covering never more than 12 per cent. of the ventral surface. This occurrence has been remarked by Little in the case of Type "B" mice resulting from the

cross black-eyed white by self. Further reference will be made to these mice in the section on modifying factors. In addition certain mice have appeared which exhibit only a small white spot in the form of a "blaze" of white hairs between the eyes which may or may not be accompanied by a small belly spot. Such mice have proved to be self in distinction from piebald since when mated *inter se* they have produced 15 self-colored mice lacking any spotting and 6 piebalds, indicating that both "blaze" parents were selfs heterozygous for piebald. When tested by crossing with pure piebalds, such "blaze" mice have produced 55 selfs and 39 piebalds. Certain of the selfs had a small white spot (always less than 10 per cent.) *ventrally*, and it is possible that these may be piebalds. If so, the ratio of 1 self:1 piebald may be more closely approached, indicating that the small white dorsal spot is a non-genetic imperfection of dominance occurring in self mice heterozygous for piebald. None of the "self" progeny from crosses of blaze with piebald exhibit any dorsal spotting. One other possibility is that the blaze may be due to a separate gene either identical with or similar to the gene which differentiated the blaze mice reported by Little. The second or other segregating generation which is critical for determining this point has not been bred. Of course the term self should apply properly only to mice which show no trace of white spotting. Genetically it is the sum of the factors producing the normal solid coat of the wild mouse and as such should be always the same unless new mutations take place or unless certain somatic variability exists unconnected with a germinal cause. Sufficient data in the case of the dorsal spotting of apparently self mice are not at present available to decide between these alternatives. In the case of the small belly spotting, as will be seen later, the case is somewhat different.

For convenience in reference the range of variability of each of the several genotypes discussed above has been placed in tabular form (Table I). All ranges except that for piebald (line 3) have been drawn from ob-

servations by the writer and all data in the tables and figures of this report have been tabulated according to these ranges. Such a table is approximate and may be only temporary, for the breeding of larger numbers of

### Summary of Range Data

TABLE I

| Type of Spotting | Genotype | Per Cent. of Dorsal White | Per Cent. of Ventral White |
|---|---|---|---|
| 1. Black-eyed white | *Wwss* | 100 to 50 | 100 to 85 |
| 2. Piebald (present series) | *wwss* | 50 to 0 | 85 to 12 |
| 3. Piebald (other data) | *wwss* | 100 to 0 | 85 to 0 |
| 4. Type A | *WwSs* | 45 to 0 | 90 to 4 |
| 5. Type C | *WwSS* | 8 to 0 | 50 to 15 |
| 6. Self | *wwSS* or *wwSs* | 3 to 0 | 12 to 0 |

spotted mice may increase the range now attributed to each type. Whether the ranges of variability represent the norm for each type is not at present known, nor can it be known until each type by inbreeding or other suitable methods has been separated from the subsidiary factors which, as it will appear, alter the expression of the main spotting factors.

### Modifying Factors

The general result of the foregoing discussion is simply an exposition of the great amount of variability existent within each type of spotted mice, all of which are identical as regards the main genes now known to determine white spotting. The question naturally arises, if a mouse with a coat entirely white except for pigment in the eyes and a mouse in which the dorsal surface is equally divided between pigmented and white spaces are genetically identical as regards the main genes *W* and *s*, how then do they differ? Is each merely a somatic variation (fluctuation) of a genetic complex determining a combination of colored and white spaces halfway between these extremes? Or do other genetic factors in combination with the main spotting genes determine greater or less amounts of pigment in the coat?

If we adopt the fluctuation hypothesis then we must

demonstrate that the amount of spotting in the offspring is not correlated with the amount of spotting in the parents. Being due to non-genetic causes, the variations within the type should appear purely at random. An excellent example of this kind of variation has been communicated in a paper read before the American Society of Naturalists at their December, 1919, meeting by Dr. Sewall Wright. The piebald pattern of guinea pigs appeared in his experiments to be determined primarily by one recessive Mendelian gene, the expression of which is altered through extremely wide ranges by environment, sex and the uncontrolled vagaries of development.

The second explanation presumes the occurrence of modifying genes separable in heredity from other genes for spotting, yet only coming to expression in the presence of the main gene or genes for spotting. If this be true, the various grades of spotting within any type such as black-eyed white should be heritable. The amount of spotting in the offspring should be definitely correlated with the amount of spotting in the parents. Whiter black-eyed whites, for example, should have whiter offspring; and darker black-eyed whites should have darker offspring. Little has found some evidence that this may be true of certain grades of piebald, for he noted that the offspring of hybrids between selfs and piebald tended to cluster about the mode of the particular piebald grandparents.

The experimental test of these diverse explanations has in the present experiments consisted of crossing mice of one spotted type with lighter (much spotted) and darker (little spotted) mice of another type. The offspring of the lighter and darker matings have then been compared as to mean and range. Some matings of lighter types *inter se* and darker types *inter se* have been made, and while these have not yet yielded enough offspring to have a decisive bearing on the question, they indicate that black-eyed white and piebalds with more white spotting than the mean of their respective types and with amounts of spotting below the mean

breed fairly true to these different conditions. The results of the first mentioned matings have shown in general that the altered expression of the spotting factors *W* and *s* in the direction of more or less white spotting is definitely transmitted to the offspring. The evidence on this point is presented in Table II.

Cross 1 of this table presents the distribution of offspring of crosses of black-eyed whites with dark piebalds, viz., those with dorsal white spotting ranging from 0–10 per cent. Among the black-eyed white parents all grades of spotting were approximately equally represented. The mode of the black-eyed white offspring is at 85–81 per cent. of white, the mean of white spotting is 80.5 per cent. $\pm$ .7 per cent., and they range from 100 to 51 per cent. white, with a standard deviation of 11.65 $\pm$ .5. The piebald young have a mean grade of 10.9 $\pm$ .4 per cent., a standard deviation of 8.1 $\pm$ 3, and all grades of piebald from 0 to 50 per cent. white are represented, although the majority is less than 20 per cent. white. With these crosses are to be compared the offspring of Cross 2, of which the parents were black-eyed whites of the same grades as were used in Cross 1, and *light* piebalds, viz., those which were more than 10 per cent. white dorsally. The black-eyed white young were centered about a mode at 95–91 per cent. white; their mean is 88.5 per cent. $\pm$ .8 per cent., and their standard deviation 7.8 $\pm$ .5. Their range is considerably less than the range of black-eyed whites in cross 1, due to the absence from the distribution of all classes less than 70–66 per cent. white. The piebald young from Cross 2 have a mean of 16.4 $\pm$ 1.2 per cent. white and a standard deviation of 12.1 $\pm$ .8. The range of the piebalds is the same as in Cross 1, but the lighter classes are more heavily represented than in Cross 1, and this is reflected in the higher standard deviation.

These crosses are a test of the nature of the differences between darker and lighter piebalds. That such differences are genetic is clearly shown by the results, for the lighter piebalds have appreciably lighter offspring

TABLE II

CROSSES OF SPOTTED MICE SHOWING VARIATION IN AMOUNT OF WHITE SPOTTING

| Cross | Per Cent. of Dorsal White on Young | | | | | | | | | | | | | | | | | | | | Self | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Black-eyed Whites | | | | | | | | | | Piebald, Type "A," and "Spotted" | | | | | | | | | | | |
| | 100–96 | 95–91 | 90–86 | 85–81 | 80–76 | 75–71 | 70–66 | 65–61 | 60–56 | 55–51 | 50–46 | 45–41 | 40–36 | 35–31 | 30–26 | 25–21 | 20–16 | 15–11 | 10–6 | 5–0 | | |
| 1. Black-eyed-white × piebald (*Wwss* × *wwss*) | 13 | 34 | 34 | 27 | 12 | 12 | 13 | 6 | 6 | 3 | 2 | 1 | 5 | 3 | 5 | 13 | 17 | 24 | 46 | 42 | 0 | 323[7] |
| 2. Black-eyed white × self (*Wwss* × *wwSS*) | | | | | | | | | | | | 1 | 1 | 2 | 0 | 2 | 1 | 6 | 1 | 26 | 36 | 76 |
| 3. Type A × piebald (*WwSs* × *wwss*) | 15 | 23 | 23 | 20 | 13 | 5 | 7 | 3 | 4 | 0 | 5 | 2 | 6 | 6 | 10 | 6 | 21 | 21 | 45 | 89 | 116 | 443[8] |
| 4. Type A × Type A (*WwSs* × *WwSs*) | 7 | 3 | 3 | | | | | 1 | 2 | | 2 | | 3 | 1 | 2 | 1 | | 1 | 6 | 11 | 18 | 61 |
| 5. Type A × self (*WwSs* × *wwSS*) | | | | | | | | | | | | | | | | | | 2 | 13 | 12 | 33 | 60 |
| 6. Type C × piebald (*WwSS* × *wwss*) | | | | | | | | | | | | | | | | 1 | 2 | 6 | 8 | 26 | 42 | 85 |
| 7. Black-eyed white × black-eyed white (*Wwss* × *Wwss*) | 1 | 1 | 3 | 2 | | 1 | 1 | 2 | 2 | | | | 1 | | | | 1 | | 1 | 1 | | 17 |
| 8. All black-eyed whites (*Wwss*) | 36 | 61 | 63 | 49 | 25 | 18 | 21 | 12 | 14 | 3 | | | | | | | | | | | | 302 |
| 9. All Type "A" (*WwSs*) | | | | | | | | | | | | 1 | 1 | 2 | 0 | 3 | 3 | 12 | 9 | 52 | | 83 |
| 10. $F_2$ piebalds from cross of piebald self × (*wwss*) | | | | | | | | | | | | 2 | 1 | 3 | 4 | 4 | 16 | 41 | 74 | 133 | | 278 |
| 11. All piebalds[9] (*wwss*) | | | | | | | | | | | 2 | 4 | 6 | 6 | 9 | 17 | 33 | 65 | 120 | 175 | | 437 |

[6] In this class are placed mice of Types A and C as well as piebalds occurring in the same distribution since these types are not distinguishable somatically.

[7] Includes 5 piebalds not graded.

[8] Includes 3 "spotted" not graded.

[9] Includes all piebalds from Cross 1, from other matings of piebald × pie bald, and of $F_2$ out of piebald × self.

TABLE II.—*Continued*

CROSSES OF SPOTTED MICE SHOWING VARIATION IN AMOUNT OF WHITE SPOTTING

| Cross | Types Observed and Expected Classified According to Ranges Given in Table I | | | | | | Mean Grade (M.) and Standard Deviation (S.D.) for the Larger Distributions | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Black-eyed White | | "Spotted"[6] | | Type A | | Piebald | |
| | | Black-eyed White | "Spotted"[6] | Type A | Piebald | Self | M. | S.D. | M. | S.D. | M. | S.D. | M. | S.D. |
| 1. Black-eyed white × piebald ($Wwss \times wwss$) | Observed | 160 | | | 163 | | | | | | | | | |
| | Expected | 161.5 | | | 161.5 | | 83.8±.6 | 11.3±.4 | | | | | 12.5±.6 | 11.2±.4 |
| 2. Black-eyed white × self ($Wwss \times wwSS$) | Observed | | | 40 | | 36 | | | | | | | | |
| | Expected | | | 38 | | 38 | | | | | 9.5±1.1 | 10.9±.9 | | |
| 3. Type A × piebald ($WwSs$ $wwss$) | Observed | 113 | 214 | | | 116 | | | | | | | | |
| | Expected | 110.7 | 221.5 | | | 110.7 | 84.7±.6 | 10.3±.5 | 11.6±.5 | 11.3±.4 | | | | |
| 4. Type A × Type A ($WwSs \times WwSs$) | Observed | 16 | 27 | | | 18 | | | | | | | | |
| | Expected | 10.2 | 35.5 | | | 15.2 | | | | | | | | |
| 5. Type A × self ($WwSs \times wwSS$) | Observed | | 27 | | | 33 | | | | | | | | |
| | Expected | | 30 | | | 30 | | | | | | | | |
| 6. Type C × piebald ($WwSS \times wwss$) | Observed | | | 43 | | 42 | | | | | | | | |
| | Expected | | | 42.5 | | 42.5 | | | | | 6.7±.5 | 5.06±.4 | | |
| 7. Black-eyed white × black-eyed white ($Wwss \times Wwss$) | Observed | 13 | | | 4 | | | | | | | | | |
| | Expected | 11.3 | | | 5.7 | | | | | | | | | |
| 8. All black-eyed whites ($Wwss$) | | | | | | | 83.5±.4 | 11.4±.3 | | | | | | |
| 9. All Type "A" ($WwSs$) | | | | | | | | | | | 7.9±.6 | 8.4±.4 | | |
| 10. $F_2$ piebalds from cross of piebald × self ($wwss$) | | | | | | | | | | | | | 8.0±.3 | 7.0±.2 |
| 11. All piebalds[9] ($wwss$) | | | | | | | | | | | | | 9.7±.3 | 8.6±.2 |

than the darker piebalds. The difference in the means of white spotting in the offspring of the two crosses is significant when the errors are considered.[4] When we consider the kinds of individuals produced, we find that the darker piebalds produce certain classes of young which the lighter piebalds do not produce. The darker piebalds appear to possess then a factor or factors determining an increase in the amount of pigment produced and a consequent decrease in the amount of the white spotting. The lighter piebalds do not give evidence of possessing these genes or if they do possess certain of them they do not at any rate possess the number or the kinds which are apparent in the darker piebalds. It is to be especially noted that such modifying genes produce effects equally on the amount of black-eyed white spotting and on the amount of piebald spotting.

In the above case the assumed modifying genes came from piebalds differing in amounts of white spotting. In crosses 3 and 4 (Table III) their effect has been tested when entering in connection with black-eyed white spotting. The spotting produced in Type "A"s (*WwSs*) must be due to the gene *W* for animals of the formula *Ss* are not spotted except for certain imperfections of dominance already noted. Darker Type "A"s (0–10 per cent. white dorsally) and lighter Type "A"s (more than 10 per cent. white) were tested by mating with piebalds of various grades ranging from 20 per cent. white to 0 per cent white. The young from darker Type "A"s × piebald (Cross 3) were of three sorts as expected, black-eyed whites, "spotted" (comprising Type "A"s and piebalds) and selfs in the approximate ratio of 1:2:1. The mean of the black-eyed whites was 81.3 ± .8 per cent. white, which is about the same as the mean of black-eyed whites out of dark piebalds. They varied from 100 per cent. to 56 per cent. white with a standard deviation of 10.3 ± .6. The "spotted" young from this cross had a mean grade of 8.6 per cent. ± .5, a range

[4] The difference in mean grade of offspring of lighter and darker parents is 8.0 ± 1.06, or more than 7 times the probable error.

TABLE III

CROSSES OF BLACK-EYED WHITE AND TYPE "A" MICE OF VARIOUS GRADES WITH PIEBALD MICE TO ASCERTAIN GENETIC SIGNIFICANCE OF VARIATION IN AMOUNT OF WHITE SPOTTING

*Distribution of Young with Various Amounts of Dorsal White*

| Cross | 100–96 | 95–91 | 90–86 | 85–81 | 80–76 | 75–71 | 70–66 | 65–61 | 60–56 | 55–51 | 50–46 | 45–41 | 40–36 | 35–31 | 30–26 | 25–21 | 20–16 | 15–11 | 10–6 | 5–0 | Self | Total | Black-eyed Whites | | | "Spotted" | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | | | | | | | | M. | S.D. | Total | M. | S.D. | Total |
| 1. Black-eyed whites of all grades × piebalds less than 10% white dorsally | 8 | 16 | 21 | 24 | 11 | 10 | 10 | 6 | 6 | 3 | 1 | 1 | 1 | 1 | 2 | 8 | 11 | 18 | 39 | 31 | | 228 | 80.5±.7 | 11.6±.5 | 115 | 10.9±.4 | 8.1±.3 | 113 |
| 2. Black-eyed whites of all grades × piebalds more than 10% dorsally | 5 | 18 | 13 | 3 | 1 | 2 | 3 | 0 | 0 | 0 | 1 | 0 | 4 | 2 | 3 | 5 | 6 | 6 | 7 | 11 | | 90 | 88.5±.8 | 7.8±.5 | 45 | 16.4±1.2 | 12.1±.8 | 45 |
| 3. Type "A's" less then 10% white dorsally × piebalds of all grades | 4 | 9 | 18 | 16 | 10 | 5 | 7 | 3 | 4 | 0 | 2 | 0 | 2 | 1 | 4 | 3 | 11 | 11 | 33 | 72 | 85 | 300 | 81.3±.8 | 10.3±.6 | 76 | 8.6±.5 | 8.9±.3 | 139 |
| 4. Type "A's" more than 10% white dorsally × piebalds of all grades | 11 | 14 | 5 | 4 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 2 | 4 | 5 | 6 | 3 | 10 | 10 | 12 | 17 | 37 | 146 | 91.7±.7 | 6.19±.5 | 37 | 17.2±1.0 | 13.2±.7 | 72 |

from 50 to 0 per cent. white and a standard deviation of $8.9 \pm .3$.

In Cross 4 the piebald parents were of similar grades to those used in Cross 3 while the Type "A" parents were all *more* than 10 per cent. white dorsally. The black-eyed white young from this cross had a mean grade of $91.7 \pm .7$ per cent. white. They varied from 100 per cent. to 76 per cent. white with a standard deviation of $6.19 \pm .5$. The "spotted" young (Type "A" and piebald mixed) were of mean grade $17.2 \pm 1.0$ per cent. white, and varied from 50 per cent. to 0 per cent. white, with a standard deviation of $13.2 \pm .7$. In general the offspring of the lighter Type "A" parents were characterized by about 10 per cent. more white spotting than the offspring of the darker Type "A" parents. The difference of the parents in amount of spotting is thence reflected in similar differences in their respective offspring.

The indications from the tests of Type "A" spotted mice are that the same modifying factors which were assumed to cause the variation in the amount of spotting in piebalds cause also the variation in the expression of the gene $W$ as evidenced in Type "A" spotting. Here also certain classes of dark black-eyed whites appear when dark Type "A"'s are bred which are absent from the young produced by lighter Type "A"'s. This absence is witnessed by the significantly lower variabilities of the black-eyed white offspring of both lighter piebald and lighter Type "A"'s. The effect of the modifiers is the opposite when acting on the piebald and spotted offspring of these crosses. The piebald offspring of lighter piebald and Type "A" parents have a *greater* range and consequently a *higher* standard deviation than the piebald offspring of darker parents. The darkening modifiers *add* darker classes to the black-eyed white range but *subtract* from the lighter classes of piebald, lowering in general the amount of white in each type. A "light" piebald, namely, one near the upper limit of piebald spotting and lacking the dark modifiers, may thus be similar in appearance to a "dark" black-eyed white.

namely, one near the lower limit of black-eyed white spotting and possessing the dark modifiers. Such confusion is not apt to occur in the progeny of single pairs, for if the parents possess the modifiers dark black-eyed whites will be produced, but also dark piebalds, leaving an appreciable gap between the two types.

## The Nature of the Modifiers

The effect of the same modifying genes upon the expression of both black-eyed white and piebald spotting furnishes certain information concerning the nature of the modifying genes themselves. The main spotting genes *W* and *s* have been found to be properties of distinct loci in different chromosomes (Dunn, 1920). Therefore the gene or genes which modify both *W* and *s* must determine the general conditions underlying the formation of pigment in the coat rather than specific conditions associated with a particular spotting gene. From the present evidence it appears to the writer that these modifying genes alter the internal environment of enzyme and chromogen upon which the main spotting genes *W* and *s* act to bring about their specific effects. The darkening modifiers appear to increase the general *amount* of color forming substance. In the presence of such modifiers both *W* and *s* produce relatively less than the normal amount of white spotting.

## The Source of the Modifiers

The genes modifying the amount of white spotting in the mice used in these experiments appear to have come from certain self mice with which black-eyed whites and piebalds had been crossed. The black-eyed white stock used was originally bred by Dr. J. A. Detlefsen and reached this laboratory through a fancier. When first bred *inter se* no grading records were kept, but I am certain that no black-eyed whites were produced which were less than 70 per cent. white. This agrees fairly well with the range of the black-eyed whites bred by Little. The

piebalds used were obtained originally from fanciers. Sixty-five such piebalds varied from approximately 50 per cent. white to 0 per cent. white dorsally, with a mean grade of about 22 per cent. white dorsally. When these had been crossed with self mice 278 piebalds extracted in the second generation averaged 8 per cent. white dorsally. Their range was approximately the same as that of the original piebalds but the darker classes between 0–10 per cent. white had been greatly enlarged. It is probably from this cross that the darkening modifiers were introduced into the piebald stock, for the piebalds used in all subsequent crosses were descended from these extracted piebalds, most of which showed but little white spotting. It might be supposed that the darkness of the extracted piebalds could be explained on the basis of contamination of the piebald gene during its residence in self animals. That this is not the true explanation is indicated by other results. The cross of black-eyed white × piebald (Table II, Cross 1) yields black-eyed white progeny with a mean grade of 82.8 per cent. and piebalds averaging 12.5 per cent. The cross Type "A" × piebald (Table II, Cross 3) yields black-eyed white young with a mean grade of 84.8 per cent. white and "spotted" young, with a mean grade of 11.6 per cent. white. The means in the two crosses do not differ sensibly. Yet Type "A" is heterozygous for self and the young it produces should exhibit contamination if any takes place. Certain of the selfs with which piebalds were crossed originally, and certain selfs with which black-eyed whites were crossed later to produce Type "A"'s appear to have contributed modifying genes which could have had no expression in the self mice, since these lacked the main spotting genes. Not all selfs are genotypically similar in respect to the modifiers, since from crosses of black-eyed whites with certain selfs has arisen a lighter strain of black-eyed white which is now being bred, while from other selfs has come a darker strain of black-eyed white which probably possesses the modifiers.

The probable existence of additional genes in mice of the nature of modifying factors has been stressed because it is felt that animals which can be easily bred in the laboratory should be thoroughly explored genetically, in order to find out characters not known at present. As the number of genes approaches the number of chromosomes, the probabilities of finding linkage become greater and it is through the investigation of linkage and the localization of the hereditary determiners that the most exact knowledge concerning the nature of the hereditary material can be secured.

## Inheritance of Belly Spotting

Before concluding this discussion of modifiers of white spotting in mice some evidence may be added which bears on the appearance of a small amount of white spotting on the bellies of mice which are genetically self as regards *W* and *s*. Piebald spotting has been regarded as the recessive allelomorph of self or uniform coloration, and all the evidence from crosses between these two varieties supports this view. It has been noticed, however, that the heterozygotes resulting in $F_1$ are not always exact duplicates of the self parents. Where large numbers have been bred, investigators have always found $F_1$ animals with some white spotting, usually consisting of a patch of white on the belly not exceeding 12 per cent. of the ventral surface.

Records of experiments in the present investigation disclosed this apparent imperfection of dominance, and all animals resulting from the cross of piebald with self have been graded according to the per cent. of white spotting exhibited. A tabulation of these records shows that out of 51 pure piebalds bred to self mice, 36 produced only self mice with no white hairs; 15 produced some perfect self and some with white hairs. Of these 15 parents only 7 produced more than one young showing any white spotting. 557 young resulted from all piebald × self matings, of which 524 showed no trace of spotting

while 33, or about 6 per cent., showed one or more white hairs. The spotting in the $F_1$ mice was confined to the ventral surface,[5] usually in the center of the belly. The minimum size of this spot was a few white hairs, its maximum extent was 12 per cent. of the ventral surface, and its average extent was 3 per cent. of the ventral surface. All young produced by this cross must be regarded as selfs since all produced young, when interbred, in the ratio of three selfs to one piebald. What, then, is the cause of the appearance of certain animals in $F_1$ which show some characteristics of the recessive parent?

There may be two answers to this question: (1) the apparent imperfections of dominance in $F_1$ may be due to fluctuations in the somatic expression of the piebald gene when present singly in the zygote; (2) they may be due to a definite gene or genes for a small amount of ventral spotting in mice heterozygous for piebald.

As evidence for the first view we have relevant data in the experiments just reported. An analysis reveals that the production of young with small amounts of white spotting is not correlated with the somatic appearance of the piebald parents since in amount and distribution of spotting these parents as a class are not distinguishable from the parents which produced only true self young. On the other hand, practically all the spotted $F_1$s which were produced by individual piebald parents had for their other parent a particular self animal. Apparently the selfs as well as the piebalds varied in the power of producing slightly spotted young. Causes influencing the production of this small amount of spotting in $F_1$ may have been contributed by the self parents, although the posibility that part of the causes came from the piebald parents is not excluded by the evidence. If this be accepted as evidence that selfs share in the production of slightly spotted mice, then certain selfs must differ genetically from other selfs, and the point of difference may be a separate recessive factor for ventral

[5] With the exception of the few dorsally spotted mice referred to on p. —.

spotting, which is expressed in the presence of but one dose of piebald.

On this view, self animals which, when bred to piebalds, produce animals with small amounts of ventral spotting, must be heterozygous for a recessive gene for such spotting. Moreover, the piebald parents must also be heterozygous in the same gene. This is required by the evidence. 15 pairs of parents producing young with some white gave a total of 151 young, of which 33, or 21 per cent., showed some white. This is nearly a ratio of three perfect selfs to one with some white, and if the cause of the small spotting is a gene, both piebald and self parents must have been heterozygous for it. The presence of such a gene in piebald mice is difficult of demonstration, since when bred together piebald mice produce only piebalds, with their characteristic dorsal and ventral spotting, which obscures the action of any genes for spotting of the ventrum only.

A summary now partially completed shows that the peculiarity of small belly spotting in "self" mice does not breed true. Matings of "belly spot" × "belly spot" have produced 70 young of which 57 were graded as self (viz., having either no white spotting or less than 12 per cent. white ventrally) while 13 were clearly piebald. Individual tests showed that all belly spotted mice bred were heterozygous for piebald, so this ratio is probably a deviation from a 3:1 ratio. Of the 57 "selfs," 41 had some ventral spotting like the parents, while 16 were true selfs without any white hairs. The appearance of these true selfs, and the fact that all belly spotted mice tested were heterozygous for piebald indicate that the assumed gene for belly spotting is only expressed by mice which are heterozygous for piebald. The total distribution from the matings just referred to resembles somewhat a 1:2:1 ratio, which would be expected if selfs heterozygous for piebald show by reason of an imperfection in dominance, a small amount of ventral spotting. The distribution tabulated on this assumption follows:

| | Self (*SS*) | Self (Belly-Spot) *Ss*+X | Piebald *ss* | Total |
|---|---|---|---|---|
| Observed | 16 | 41 | 13 | 70 |
| Expected | 17.5 | 35 | 17.5 | 70 |

That the variation is inherited is argued by the much greater frequency of belly spotted individuals in the offspring of parents showing the characteristic than in the total progeny of all self × piebald matings. In the progeny of belly spotted mice 71 per cent. of all selfs were belly spotted, while of all selfs heterozygous for piebald only 6 per cent. were belly spotted. The problem is doubtless complicated (as are probably all spotting problems) by the occurrence of a certain amount of uncontrollable somatic variation in the expression of the genes, by reason of which the truly genetic variations cannot always be isolated with certainty. In addition the last mentioned modifier seems to be dependent for expression on a particular complex of genes, namely, the presence in one individual of one dose of piebald and one of its normal allelomorph (*Ss*).

## Summary of Modifiers of Amount of White Spotting

1. The expresson of the complex of genes producing black-eyed white spotting (*Wwss*) is subject to modification by a gene or genes determining an increased amount of pigment and a decreased amount of white spotting. The normal range of black-eyed whites being from 100 to 70 per cent. white dorsally, the addition of such darkening modifiers decreases the mean amount of white spotting in such a fashion that the range is extended to as low as 50 per cent. white.

2. The expression of the gene for piebald spotting is subject to modification in the same direction and by the same gene or genes which modify the expression of black-eyed white. When these darkening modifiers are present in mice pure for piebald (*ss*) most of the mice are

about 10 per cent. or less white dorsally, while those with larger percentage of white are much rarer than among piebalds lacking the darkening modifiers.

3. The genotype *Ss* ordinarily produces the self coat, but in the presence of an additional modifying gene produces a small amount of white spotting on the ventral surface, varying in size from a few white hairs to 12 per cent. of that surface.

## Modifiers Affecting Localization of White Spotting in Piebald Mice

The presence of separate genes for certain localizations of spotting in piebald mice has been suspected for some time. Fanciers, for instance, have given separate names to such types as "Dutch belted" mice and have claimed that they bred true to this condition, which resembles the Dutch belted pattern of cattle. Consequently when piebald mice showing distinct localization of white spotting in the pelt have been born in these experiments they have been saved for further study.

The most striking of these localized spotting types has appeared sporadically in the piebald stock. From its appearance I have called it "white face" although it varies from a small blaze of white on the forehead to a white spot which covers the whole head back as far as the ears. The belly is spotted with white as in ordinary piebalds. The strain of white face which has been isolated breeds true to this condition, 8 matings of white-face by white face having produced 46 young, all of them white faced. The offspring of one pair of white faced mice which were brother and sister, have been inbred, brother to sister, for four generations and there have been born in these matings to date 36 young, all white faced, and varying but little in the amount and distribution of the white spotting on the face.

One other sub-type of piebald is perhaps also separable. This is the type known as belted. It varies

from a wide white belt covering all of the back from shoulders to hind limbs (about 45 or 50 per cent. of the dorsal surface) down to a small spot located likewise on the back within this same area. The rest of the dorsum including the face is colored. The belly is spotted with white as in ordinary piebalds. Several good examples of this type have been saved and a few matings have been made recently. Only two matings of belted by belted have produced young. All of these young (four in number) are belted, with no spotting elsewhere on the dorsum. This is hardly a sufficient test of the separateness of this type, but more data are being accumulated.

It seems fairly evident that the production of the pattern of piebald mice is due to a complex of genes modifying the expression of one main gene. It is probable that each such gene in the complex determines the non-development of pigment in one part of the pelage. As a means of testing these hypotheses and of separating, if present, the various causative factors, the inbreeding method advanced by East warrants a trial as the most likely to bring results. Piebald mice of several different types should be inbred and the inbreeding continued in the various lines, brother to sister, for seven or eight generations. This should result in the purification of the types by the elimination of heterozygotes, and the resulting pure recessives should exhibit, if present, the effects of the separate factors. Such inbreeding is now under way on the white face and on the belted types.

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# STRUCTURAL CHARACTERISTICS OF THE HAIR OF MAMMALS

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THE microscopic structures in the hairs of mammals offer certain definite and unchanging characteristics which have been found useful for the purposes of identification.[1] The present paper aims to be an answer to numerous inquiries which the writer has received regarding: (1) the structure of a large number of mammal hairs, with especial reference to the possibility of systematically classifying them upon some morphologically accurate basis; (22) the relationships between the various elemental structures of the hair shaft; and (3) the methods employed in the preparation of the hairs for microscopic analysis.

The primary development of the hair begins as a localized proliferation of the cells of the outermost layer of the skin, known as the epidermis, forming a dense aggregation of cells which elongates downward into the corium, or dermis, beneath. Directly underneath this downward-elongated, flask-shaped depression of the cells of the epidermis there is formed a dense mass composed of cells of the corium, or dermis, which ultimately becomes the papilla of the hair (*P*, Fig. 178). The flask-shaped depression now becomes lined with cells of the epidermis, and is called the follicle. The epithelial contents of the growing follicle elongate into an avial strand of fusiform, spindle cells, which later undergoes keratinization, or becomes horny, and forms the hair shaft. The lower portion of the shaft expands into a bulb which en-

[1] Hausman: (1) "The Microscopic Identification of Commercial Fur Hairs," *Scientific Monthly*, Jan., 1920, pp. 70–78; (2) "A Micrological Investigation of the Hair Structure of the Monotremata," *Am. Journal of Anatomy*, Sept., 1920, (3) "The Microscopic Identification of Mammal Hairs Used in the Textile Industry," *The Scientific American*, Feb. 21, 1920.

wraps the papilla (Fig. 178). The shaft elongates upward, and emerges through the epidermis, an aperture thereafter known as the mouth of the follicle, and continues to grow, the growth being exclusively confined to the bulbous lower, or proximal portion of the shaft. Here the conversion of matrix cells into keratinized hair shaft cells continually progresses. Mammal hairs are in general either circular or elliptical in cross section.[2] Those which are circular are straight, or but slightly curved, while those of elliptical cross section are curly or kinky, the amount of curl being dependent upon the flatness of the ellipse.

The hair shaft consists of four structural units (Figs. 167 and 168): (1) the *medulla,* sometimes termed the pith, from a somewhat analogous structure in plant stems, and which is built up of many shrunken and variously disposed cells or chambers, representing dried and cornified epithelial structures connected by a branching filamentous network, which sometimes completely fills the medullary column, but which is interrupted in many cases; (2) the *cortex,* or shell of the hair shaft, surrounding the medulla, and composed of elongate, fusiform cells or hair-spindles, coalesced together into a horny, almost homogeneous, hyaline mass and forming in many cases, where the medulla is reduced, a large proportion of the hair shaft; (3) the *pigment granules,* to which the color of the hair is primarily due (though in some hairs the pigment is diffuse and not in granular form), scattered about within or between the hair spindles, and in some hairs arranged in definite patterns; and (4) the *cuticle,* or outermost integument of the hair shaft, lying upon the cortex, and composed of imbricated, thin, hyaline, colorless scales of varying forms and dimensions. It is the forms, relationships, and measurements of these four elements, together with the measurements of the diam-

[2] The pioneer work in the relation of the shape of the cross section of human hair in its waviness to Dr. Pruner-Bey's "De La Chevelure comme Characteristique des Races Humaines, d'après des Recherches Microscopiques," in *Mémoires de la Société d'Anthropologie de Paris,* Vol. 2, p. 1.

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eter of the hair shaft itself, in micra[3] which constitute the series of determinative criteria for each species of hair.

Medullas can be conveniently grouped, according to their forms, as they: (1) *discontinuous,* as in the hair of the Botta's pocket gopher (*Thomomomys bottæ*) (Fig. 3); (2) *continuous,* as in the hair of the kinkajou (*Cercoleptes caudivolvulus*) (Fig. 7); and (3) *fragmental,* as in the hair of the wombat (*Phascolomys ursinus*) (Fig. 64).

The cuticular scales fall readily into two well-marked types, the: (1) *imbricate,* represented in the hair of the civet (*Arctogalidia fusca*) (Fig. 1); and (2) *coronal,* represented in the hair of the majority of the bats, *e.g.,* the mastiff bat (*Molossus sinaloæ*) (Fig. 105).

The cortex element of the hair shaft structure exhibits few or no traces of the form of its component fusiform

EXPLANATION OF PLATE I

Fig. 1. Civet (*Arctogalidia fusca*), 21.00 μ.
Fig. 2. Pocket Kangaroo Rat (*Dipodomys m. nitratus*), 12.00 μ.
Fig. 3. Botta's Pocket Gopher (*Thomomys bottæ*), 25.50 μ.
Fig. 4. Coypu Rat (*Myocastor coypus*), 11.00 μ.
Fig. 5. Black Lemur (*Lemur makaka*), 20.00 μ.
Fig. 6. Chimpanzee (*Anthropopithecus troglodytes*), 119.00 μ.
Fig. 7. Kinkajou (*Cercoleptes caudivolvulus*), 34.00 μ.
Fig. 8. Rocky Mt. Jumping Mouse (*Zapus princeps*), 20.00 μ.
Fig. 9. Sierra Jumping Mouse (*Zapus trinotatus alleni*), 17.00 μ.
Fig. 10. Orolestes (*Orolestes obscurus*), 10.00 μ.
Fig. 11. Cacamixtli (*Bassariscus astutus flavus*), 17.00 μ.
Fig. 12. Striped Bandicoot (*Perameles bougainvillei bougainvillei*), 17.00 μ
Fig. 13. European Mole (*Talpa europæa*), 17.00 μ.
Fig. 14. Platypus (*Ornithorhynchus anatinus*), 8.00 μ.
Fig. 15. Star Nosed Mole (*Condylura cristata*), 25.50 μ.
Fig. 16. Pigmy Flying Phalanger (*Acrobates pygmæa*), 17.00 μ.
Fig. 17. Black Bear (*Ursinus americanus*), 27.00 μ.
Fig. 18. Red Kangaroo (*Macropus rufus*), 25.50 μ.
Fig. 19. Microgale (*Microgale dobsoni*), 18.80 μ.
Fig. 20. Aye aye (*Chiromys madagascariensis*), 24.00 μ.
Fig. 21. Koala (*Phascolarctos cinereus*), 20.40 μ.
Fig. 22. Dormouse (*Muscardinus pulcher*), 17.00 μ.
Fig. 23. House Mouse (*Mus musculus*), 17.00 μ.
Fig. 24. Gymnura (*Gymnura gymnura gymnura*), 19.00 μ.
Fig. 25. Woodland Jumping Mouse (*Napeozapus insignis insignis*), 21.00 μ
Fig. 26. Loir (*Glis glis glis*), 30.00 μ.
Fig. 27. Rat (*albino*) (*Mus norvegicus*), 17.50 μ.
Fig. 28. Hoy's Shrew (*Microsorex hoyi*), 18.00 μ.

[3] One micron (μ) is 1/1,000 of a millimeter, or circa 1/254,000 of an inch.

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cells, or hair spindles, except under dissociative treatment with caustic soda, caustic potash, or acids of various sorts, and hence is of very little value as a criterion for determining the species of the hair.

The coloring matter, or pigment, of the hair shaft is either distributed diffusely and homogeneously throughout the cortex, or exists as an aggregation of granules between or within the fusiform cortical cells, or hair tween or within the fusiform cortical cells, or hair spindles. Where the latter is the case the granules appear to be of definite form and mode of placentation for each species of hair. In many cases, it is believed that the characteristic patterns formed by the arrangement of the pigment granules, as well as the form of the granules themselves may offer a valuable character for identification. Figs. 190, 191, and 192 show respectively portions of the hair shafts of the mandril (*Cyanocephalus maimon*), badger (*Taxidea americana*), and wolverene (*Gulo luscus*), very highly magnified, illustrative of the differences which may exist in the configuration and ar-

EXPLANATION OF PLATE II

FIG. 29. Geogale (*Geogale aurita*), 12.00 μ.
FIG. 30. Potamogale (*Potamogale velox*), 10.10 μ.
FIG. 31. Golden Mole (*Amblysomus corriæ*), 13.00 μ.
FIG. 32. Heliophobius (*Heliophobius kapiti*), 15.00 μ.
FIG. 33. Marsh Shrew (*Neosorex palustris navigator*), 11.30 μ.
FIG. 34. Rock Runner (*Petrodromus tetradactylus*), 37.00 μ.
FIG. 35. Speke's Jumping Mouse (*Pectinator spekii*), 25.00 μ.
FIG. 36. Walrus (*Trichechus rosmarus*).
FIG. 37. American Wapiti (*Cervus canadensis*), 94.00 μ.
FIG. 38. Mongoose Lemur (*Lemur mongoz*), 20.00 μ.
FIG. 39. Colugo (*Galeopithecus volans*), 20.50 μ.
FIG. 40. Coendou (*Coendou sanctæmartiæ*), 25.00 μ.
FIG. 41. Flying Squirrel (*Sciuropterus volucella*), 11.70 μ.
FIG. 42. Bactrian Camel (*Camelus bactrianus*), 34.00 μ.
FIG. 43. Tiger (*Felis tigris*), 68.00 μ.
FIG. 44. Bruce's Dassie (*Procavia brucei rudolphi*), 22.00 μ.
FIG. 45. Dassie (*Procavia capensis*), 25.50 μ.
FIG. 46. Coendou (*Coendou mexicanus*), 38.00 μ.
FIG. 47. Proboscis Monkey (*Nasalis larvatus*), 47.60 μ.
FIG. 48. Spider Monkey (*Ateles geoffroi*), 32.00 μ.
FIG. 49. Great Gray Kangaroo (*Macropus giganteus*), 25.50 μ.
FIG. 50. Common Dasyure (*Dasyurus viverrinus*), 17.00 μ.
FIG. 51. Capybara (*Hydrochœrus capybara*), 34.00 μ.
FIG. 52. Small Three-Spined Tenrec (*Hemicentetes variegatus*), 28.00 μ.
FIG. 53. Unau (*Cholœpus capitalis*), 68.00 μ.
FIG. 54. European Porcupine (*Hystrix cristata*), 140.00 μ.
FIG. 55. Spiny Anteater (*Tachyglossus hystrix*), 103.00 μ.
FIG. 56. Agouti (*Dasyprocta urucuna*), 150.00 μ.

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rangement of the pigment granules. There seems to be also a wide variation in color value and color depth of the pigment granules, a variation which is especially well brought out by the use of reflected light, or of dark field illumination. These methods of examination will be explained later.

In a recent contribution to the structure of the mammalian hair[4] the author has pointed out that mammal hairs may be conveniently classified, on the basis of the configuration of the cuticular scales and medulla, as follows:

### Cuticular Scales

#### I. *Imbricate*

1. Ovate, represented by Figs. 1 to 7
2. Acuminate, represented by Figs. 8 to 20
3. Elongate, represented by Figs. 21 to 35
4. Crenate, represented by Figs. 36 to 67
5. Flattened, represented by Figs. 68 to 92

EXPLANATION OF PLATE III

Fig. 57. African Elephant (*Loxodonta africana capensis*), 80.00 μ.
Fig. 58. Ethiopian Aard Vark (*Orycteropus æthiopicus*), 252.00 μ.
Fig. 59. Hyena (*Hyæna hyæna schillingsi*), 122.00 μ.
Fig. 60. Richard's Seal (*Phoca richardi*), 232.00 μ.
Fig. 61. Two-Horned Rhinoceros (*Diceros bicornis bicornis*), 147.00 μ.
Fig. 62. Brush-Tailed Porcupine (*Ætherura africana*), 50.00 μ.
Fig. 63. Long-Tailed Pangolin (*Manis macrura*), 181.00 μ.
Fig. 64. Wombat (*Phascolomys ursinus*), 76.50 μ.
Fig. 65. Cape Aard Vark (*Orycteropus capensis*), 216.00 μ.
Fig. 66. Dinomys (*Dinomys brannicki*), 120.00 μ.
Fig. 67. Wild Boar (*Sus scrofa*), 680.00 μ.
Fig. 68. Two-Toed Anteater (*Cyclothurus didactylus*), 17.00 μ.
Fig. 69. Black-Faced Bat (*Melanycteris melanops*), 10.00 μ.
Fig. 70. Small Long-Tongued Fruit Bat (*Macroglossus minimus*), 13.00 μ.
Fig. 71. Chevrotain (*Tragulus boreanus*), 51.00 μ.
Fig. 72. Llama (*Lama glama*), 32.00 μ.
Fig. 73. Fox Terrier, 98.60 μ.
Fig. 74. Ingraham's Hutia (*Capromys ingrahami*), 76.50 μ.
Fig. 75. Jersey Cow, 42.50 μ.
Fig. 76. American Bison (*Bison americanus*), 77.00 μ.
Fig. 77. Manatee (*Manatus latirostris*), 136.00 μ.
Fig. 78. Pinche (*Midas oedipus*), 40.00 μ.
Fig. 79. Boschbok (*Tragelephus sylvaticus*), 119.00 μ.
Fig. 80. Sumatran Chevrotain (*Tragulus napu*), 55.70 μ.
Fig. 81. Hispid Pocket Mouse (*Perognathus hispidus*), 127.00 μ.
Fig. 82. Squirrel Monkey (*Chrysothrix sciurea*).
Fig. 83. Agouti (*Dasyprocta variegata*), 127.50 μ.
Fig. 84. Tamandua (*Tamandua tetradoctyla etensis*), 85.00 μ.

[4] Hausman: "A Micrological Investigation of the Hair Structure of the Monotremata," *Am. Journal of Anatomy*, Sept., 1920.

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### II. *Coronal*

1. Simple, represented by Figs. 93 to 102
2. Serrate, represented by Figs. 103 to 107
3. Dentate, represented by Figs. 108 to 113

## Medullas

### I. *Discontinuous*

*A*. Simple

1. Ovate, represented by Figs. 114 to 126
2. Elongate, represented by Figs. 127 to 128
3. Flattened, represented by Figs. 129 to 135

*B*. Compound

1. Ovate, represented by Fig. 136
2. Flattened, represented by Fig. 137

### II. *Continuous*

1. Nodose, represented by Figs. 138 to 147
2. Homogeneous, represented by Figs. 148 to 153

### III. *Fragmental*

Represented by Figs. 115 to 166

## EXPLANATION OF PLATE IV

Fig. 85. Gorilla (*Gorilla gorilla*), 37.40 μ.
Fig. 86. Virginia Deer (*Odocoileus americanus*).
Fig. 87. Sifaka (*Propithecus coronatus*), 30.00 μ.
Fig. 88. Woolly Monkey (*Lagothrix infumatus*), 30.00 μ.
Fig. 89. Orang (*Simia satyrus*), 144.50 μ.
Fig. 90. Agouti (*Dasyprocta fuliginosa*), 190.00 μ
Fig. 91. Barbirussa (*Barbirussa alfurus*), 93.00 μ.
Fig. 92. Peccary (*Dicotyles tajuca*), 407.00 μ.
Fig. 93. Yapock (*Chironectes panamensis*), 11.30 μ.
Fig. 94. Natalus (*Natalus mexicanus*), 11.00 μ.
Fig. 95. Porto-Rican Bat (*Chilonycteris parnelli portoricensis*), 8.50 μ.
Fig. 96. Hammer-Headed Bat (*Eupomorphorus anurus*), 11.00 μ.
Fig. 97. Bicolored Leaf-Nosed Bat (*Hipposiderus fulvus*), 8.50 μ.
Fig. 98. Indian Vampire Bat (*Lavia frons*), 12.00 μ.
Fig. 99. Leaf-Nosed Bat( *Rhinolophus hainanus*), 10.00 μ.
Fig. 100. Horseshoe Bat (*Rhinolophus acuminatus*), 10.00 μ.
Fig. 101. Pipistrelle (*Pipistrellus subflavus*), 6.80 μ.
Fig. 102. Java Vampire Bat (*Petalia capensis*), 10.00 μ.
Fig. 103. Cape Mole Rat (*Tachyoryctes rex*), 17.00 μ.
Fig. 104. Phyllops (*Phyllops falcatus*), 10.00 μ.
Fig. 105. Mastiff Bat (*Molossus sinaloae*), 9.00 μ.
Fig. 106. Wrinkled-Lipped Bat (*Nyctinomus bocagei*), 8.500 μ.
Fig. 107. Intermediate Bat (*Mormops intermedia*), 6.80 μ.
Fig. 108. Chief Pika (*Ochotona princeps*), 13.60 μ.
Fig. 109. Pika (*Ochotona figginsi*), 11.30 μ.
Fig. 110. Pika (*Ochotona wardi*), 11.30 μ.
Fig. 111. Alpine Chinchilla (*Lagidium pernarum*), 11.30 μ.
Fig. 112. Little Banded Anteater (*Myrmecobius fasciatus*), 20.40 μ.

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The hair type chosen to be shown as the most representative of each species is that type which, it was found, in most cases constitutes the major portion of the body covering, *i.e.*, the *fur*, or *under hair*. This usually underlies a comparatively more or less sparse growth of longer, coarser, stouter hair, which is termed the *protective*, or *over hair*. In typically aquatic mammals, such as the seals, walruses, etc., the protective hair is thicker than in those forms which are merely amphibious, such as the platypus, muskrats, beavers, etc. In such mammals as the whales, porpoises, etc., which are wholly aquatic, the fur hair has apparently vanished altogether. The only remaining hairs upon the body are, as a rule, confined to a very few stout stubs of hairs, located commonly in the region about the muzzle. In such hairs the cuticular scales are always of one type, illustrated by the muzzle hair of the dugong (*Dugong dugong*) (Fig. 159).

In identification, however, it is sometimes necessary to prepare for examination shafts of both the fur and the

EXPLANATION OF PLATE V

FIG. 113. European Otter (*Lutra vulgaris*), 10.00 μ.
FIG. 114. Chinchilla (*Chinchilla lanigera*), 16.00 μ.
FIG. 115. Dusky-Handed Tarsier (*Tarsius fuscus*), 13.00 μ.
FIG. 116. Elephant Shrew (*Macroscelides proboscideus*), 20.00 μ.
FIG. 117. Red Squirrel (*Sciurus hudsonicus*), 17.00 μ.
FIG. 118. Sewellel (*Aplodontia rufa*), 17.00 μ.
FIG. 119. Marsupial Mole (*Notoryctes typhlops*), 17.00 μ.
FIG. 120. Galeopterus (*Galeopterus gracilis*), 22.00 μ.
FIG. 121. Beecroft's Scale-Tailed Squirrel (*Anomalurus beecrofti*), 18.00 μ
FIG. 122. Viscacha (*Lagostomus maximus*), 41.00 μ.
FIG. 123. Black-Footed Ferret (*Putorius nigripes*), 20.40 μ.
FIG. 124. Nail-Tailed Wallaby (*Onychogale unguifera*), 18.50 μ.
FIG. 125. Foussa (*Cryptoprocta ferox*), 22.00 μ.
FIG. 126. Cavy (*Dolichotis salinicola*), 34.00 μ.
FIG. 127. Peters' Shrew (*Rhyncocyon petersi*), 26.00 μ.
FIG. 128. Racoon (*Procyon lotor*), 20.00 μ
FIG. 129. Philippine Tarsier (*Tarsius philippinensis*), 18.00 μ.
FIG. 130. Great Mole Rat (*Spalax typhlus*), 17.00 μ.
FIG. 131. Idiurus (*Idiurus zenkeri*), 9.10 μ.
FIG. 132. Nelson's Hare (*Romerolagus diazi*), 18.00 μ.
FIG. 133. Gray Rabbit (*Lepus nutalli mallurus*), 17.00 μ.
FIG. 134. Southern Varying Hare (*Lepus americanus virginianus*), 17.00 μ
FIG. 135. Black-Eared Marmoset (*Hapale jacchus*), 25.50 μ.
FIG. 136. Sennett Kangaroo Rat (*Perodipus sennetti*), 40.80 μ.
FIG. 137. Degu (*Octodon degus*), 34.00 μ.
FIG. 138. Agouta (*Solenodon paradoxus*), 83.00 μ.
FIG. 139. Canada Lynx (*Lynx canadensis*), 19.00 μ.
FIG. 140. European Hedgehog (*Erinaceus europeus*), 85.00 μ.

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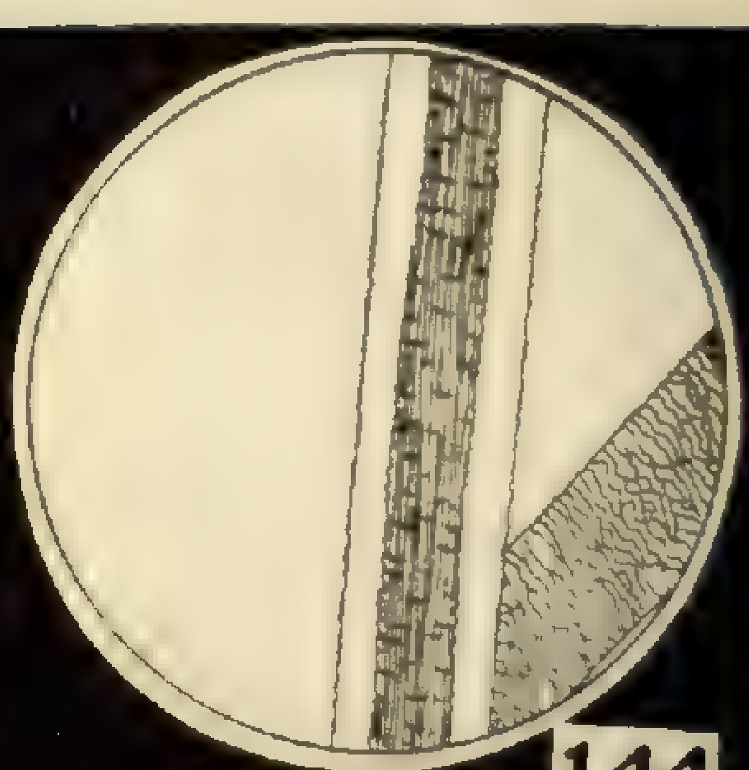
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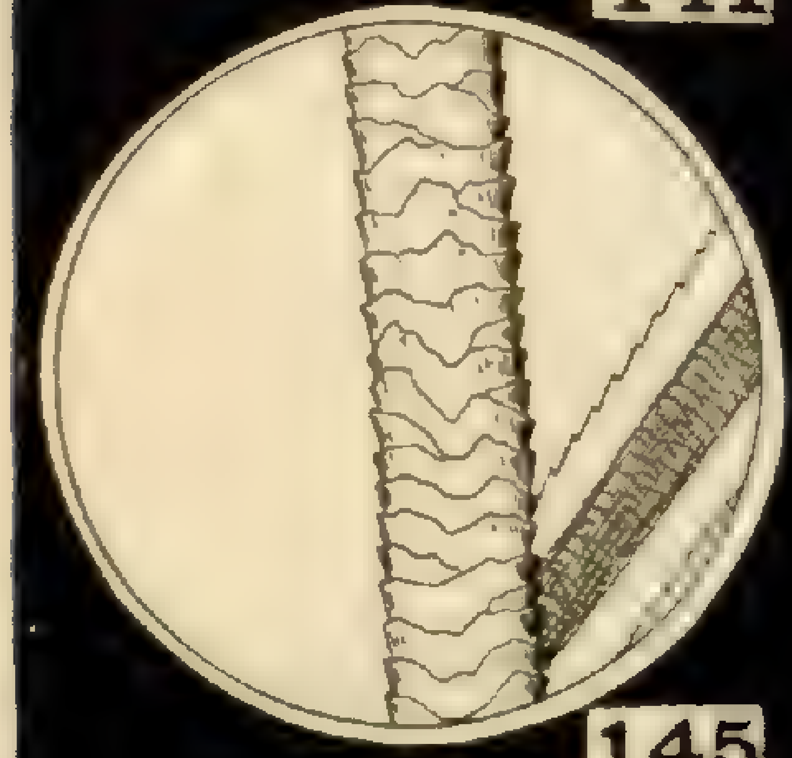
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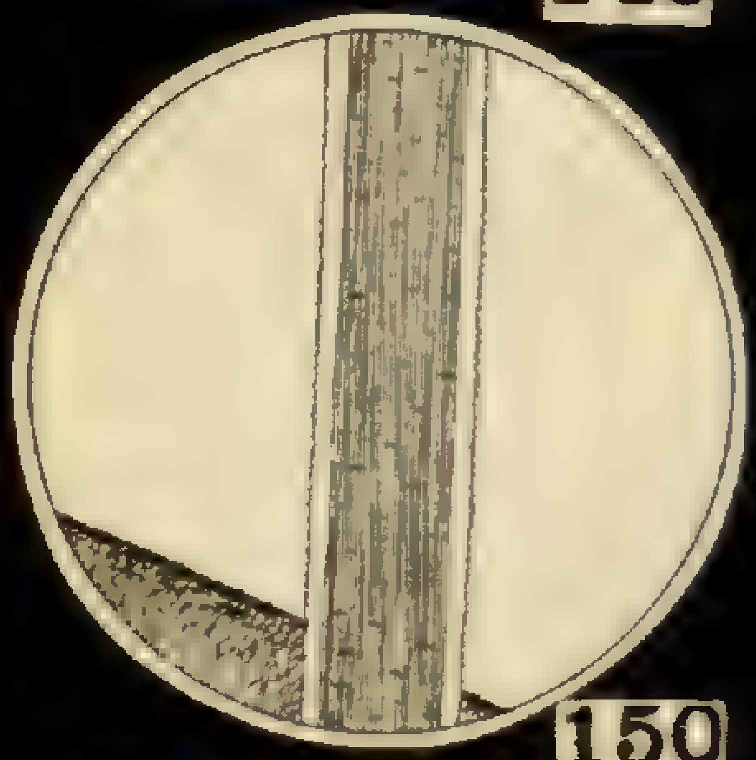
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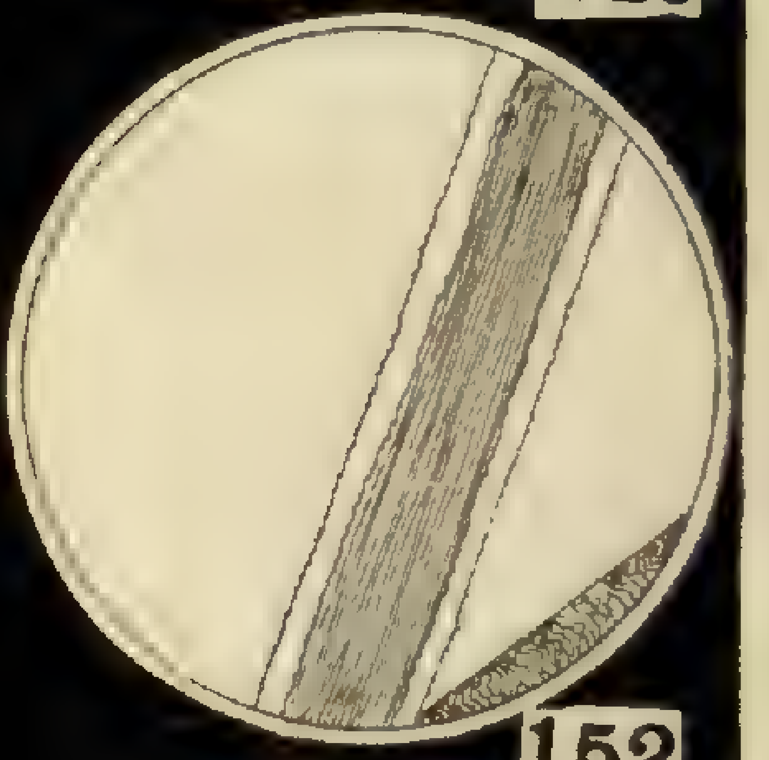
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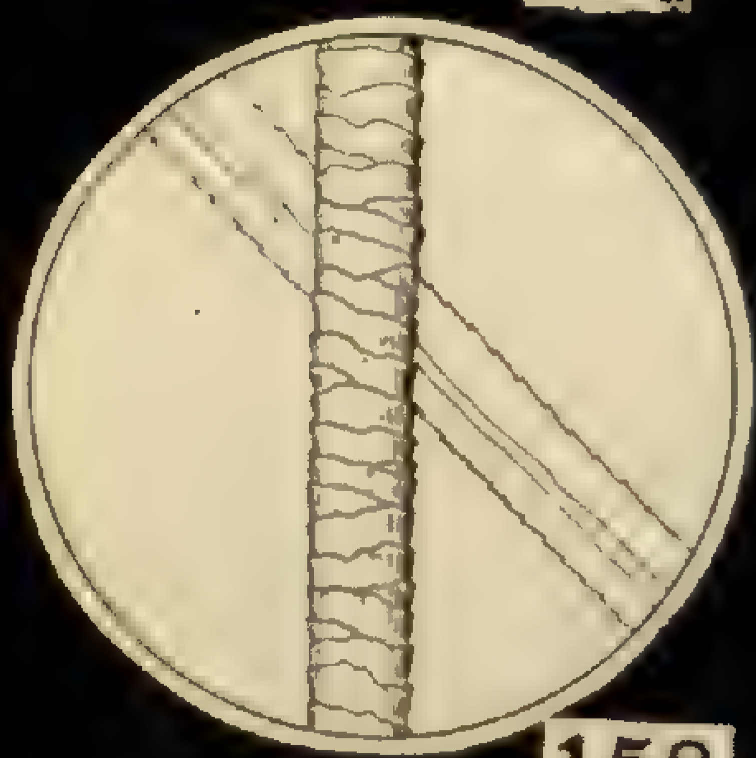
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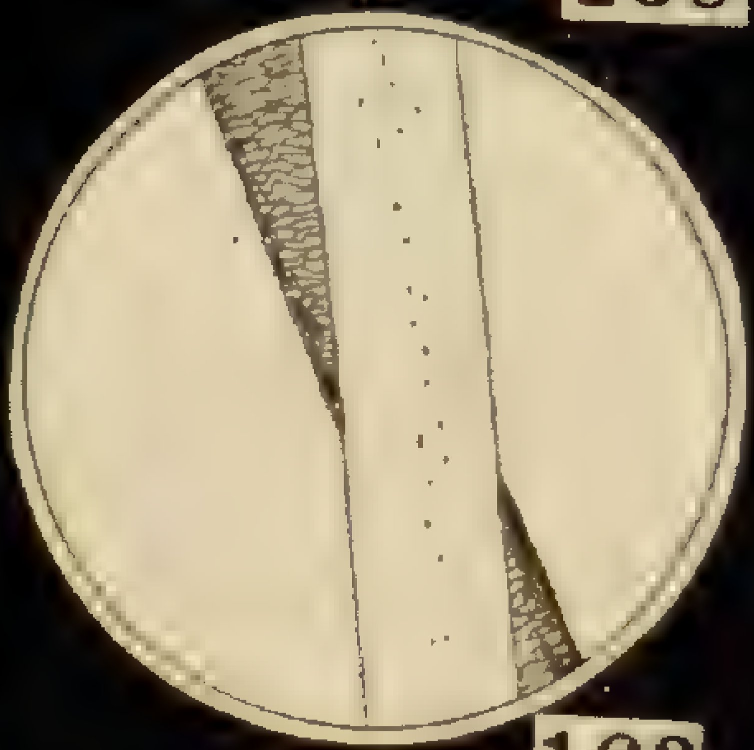
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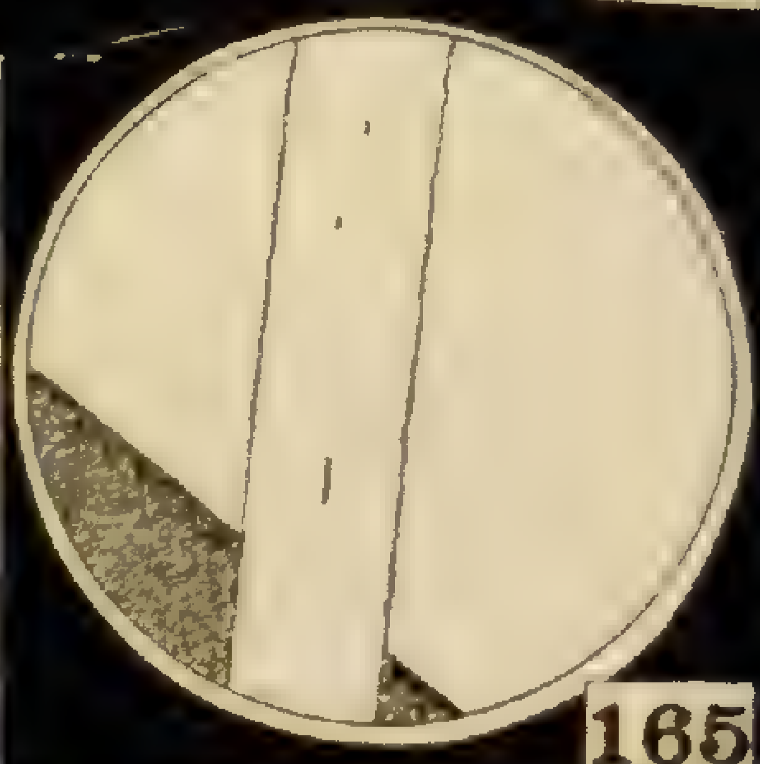
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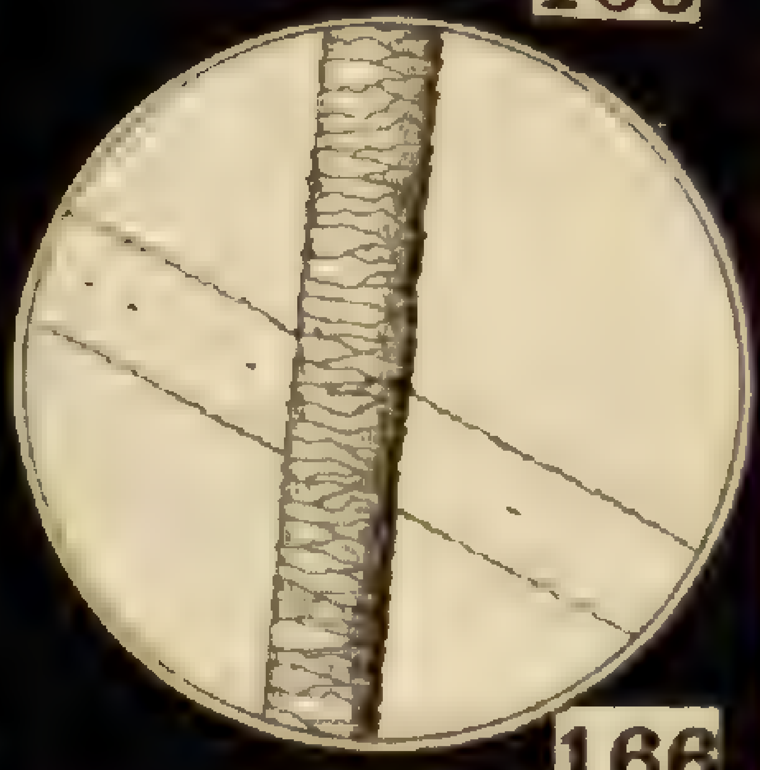
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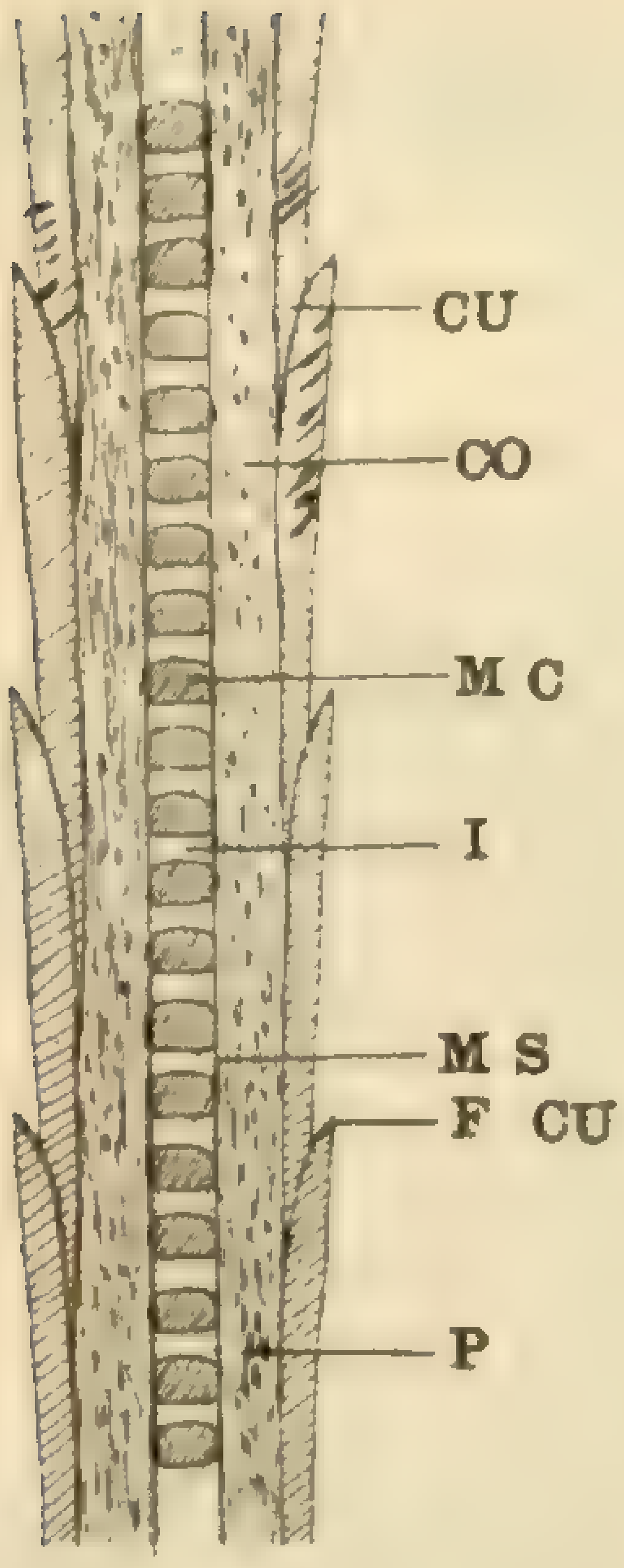
protective hair. And since the structural elements in these two types of hair usually differ considerably, a greater number of distinctive characters is thus available for comparison. However, the greater thickness and deeper pigmentation of the protective hair shafts make them much more difficult to work with than the finer, clearer fur hairs. Moreover, the scales of the protective hair are often worn off to such an extent as to make them also valueless as identification criteria. Figs. 175 to 177, and 169 to 171 show, represented to scale, the structure of the scales and medulla of the fur and protective hair of the skunk (*Mephitis mephitica*), and the European beaver (*Castor fiber*). The protective hair of mammals in general, in most cases, bears cuticular scales of the flattened or crenate type, and medullas of the continuous nodose or continuous homogeneous type.

In identifying hair species it is necessary to compare the scales and medulla from the same parts of the hairs[5]

EXPLANATION OF PLATE VI

FIG. 141. Polar Bear (*Thalarctos maritimus*), 68.00 μ.
FIG. 142. Hyena (*Hyæna hyæna bergeri*), 157.00 μ.
FIG. 143. Old World Tapir (*Tapirus terrestris*), 104.00 μ.
FIG. 144. American Tapir (*Tapirus americanus*), 74.00 μ.
FIG. 145. Langu (*Colobus caudatus matschei*), 88.00 μ.
FIG. 146. Central American Tapir (*Elasmognathus bairdi*), 96.00 μ.
FIG. 147. Rush Mouse (*Thryonomys gregorianus*), 165.00 μ.
FIG. 148. Hoffman's Sloth (*Cholœpus hoffmanni*), 68.00 μ.
FIG. 149. Ass (*Equus asinus*), 70.00 μ.
FIG. 150. Water Deer (*Hyomoschus aquaticum*), 122.00 μ.
FIG. 151. Thompson's Gazelle (*Gazella thompsoni nasalis*), 105.40 μ.
FIG. 152. Cape Giraffe (*Giraffa capensis*).
FIG. 153. Quagga (*Equus quagga bohmi*), 166.00 μ.
FIG. 154. Mongoose (*Helogale hirtula ahlselli*), 24.00 μ.
FIG. 155. Wart Hog (*Phacochœrus æthiopicus*), 357. 00 μ.
FIG. 156. Aard Wolf (*Proteles cristata*), 22.00 μ.
FIG. 157. Almiqui (*Solenodon cubanus*), 80.00 μ.
FIG. 158. Syrian Dassie (*Procavia syriacus*), 31.00 μ.
FIG. 159. Dugong (*Dugong dugong*), 1177.00 μ.
FIG. 160. Hair Seal (*Otaria jubata*), 105.00 μ.
FIG. 161. Indian Elephant (*Elephas indicus*), 200.00 μ.
FIG. 162. Coendou (*Cœndou mexicanus*), 38.00 μ.
FIG. 163. Antarctic Seal (*Hydrurga leptomyx*), 185.00 μ.
FIG. 164. Vicuna (*Lama vicuna*), 11.00 μ.
FIG. 165. Malayan Pangolin (*Manis javanica*), 290.00 μ.
FIG. 166. Mammoth (*Elephas primigenius*), from Alaska, 50.00 μ.

[5] The fur hair of many species of mammals varies upon different parts of the body, sometimes with respect to the configuration of the scales and medulla. Hence samples for comparison must be taken, as far as possible, from the same regions.

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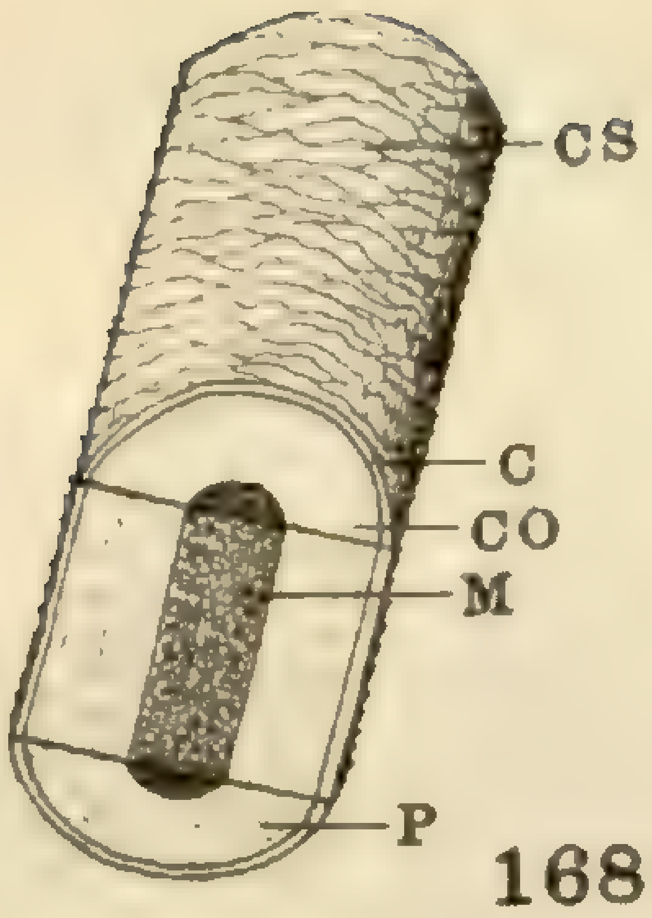


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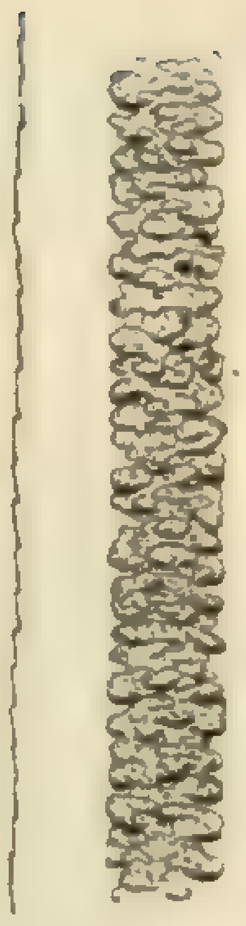

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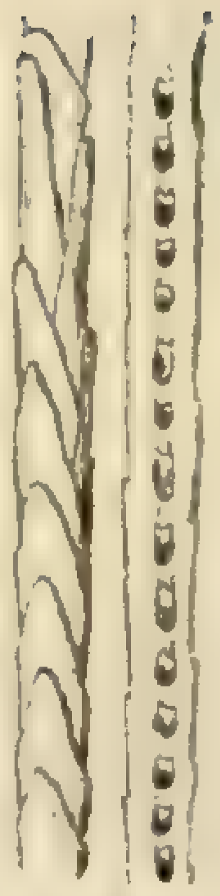

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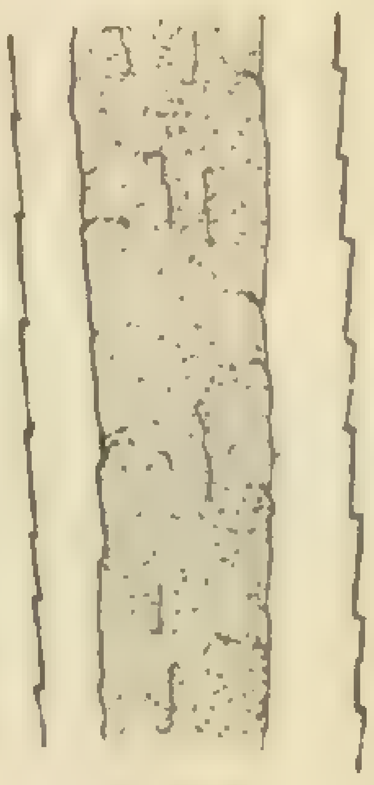

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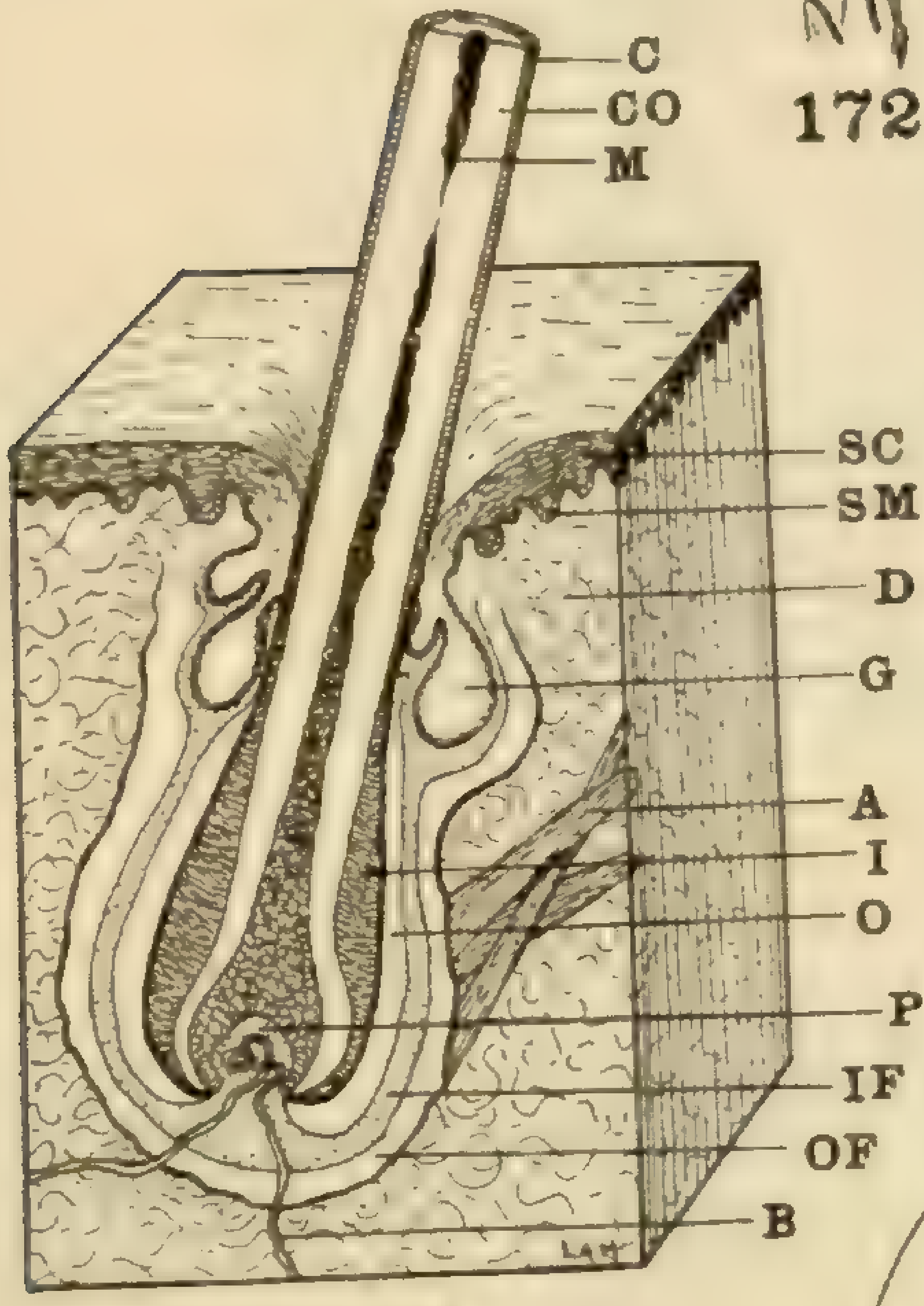


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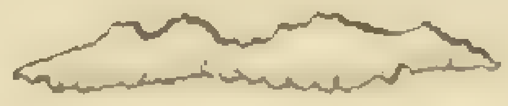

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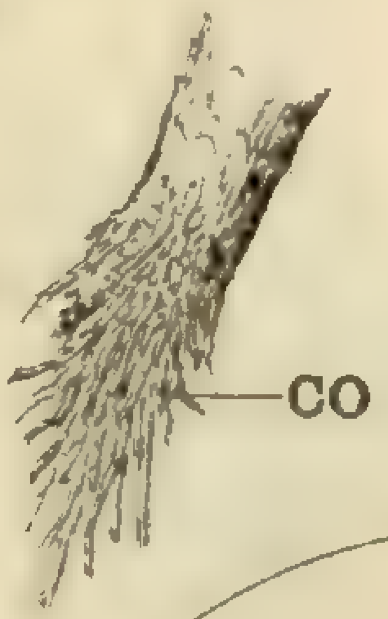


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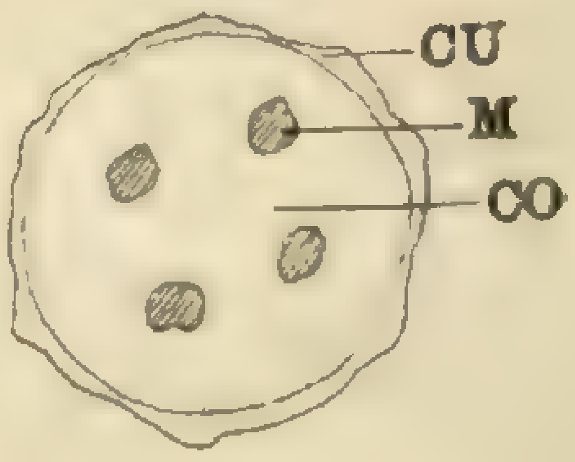


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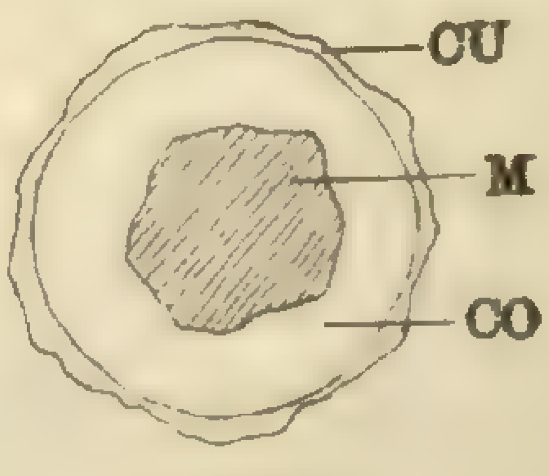


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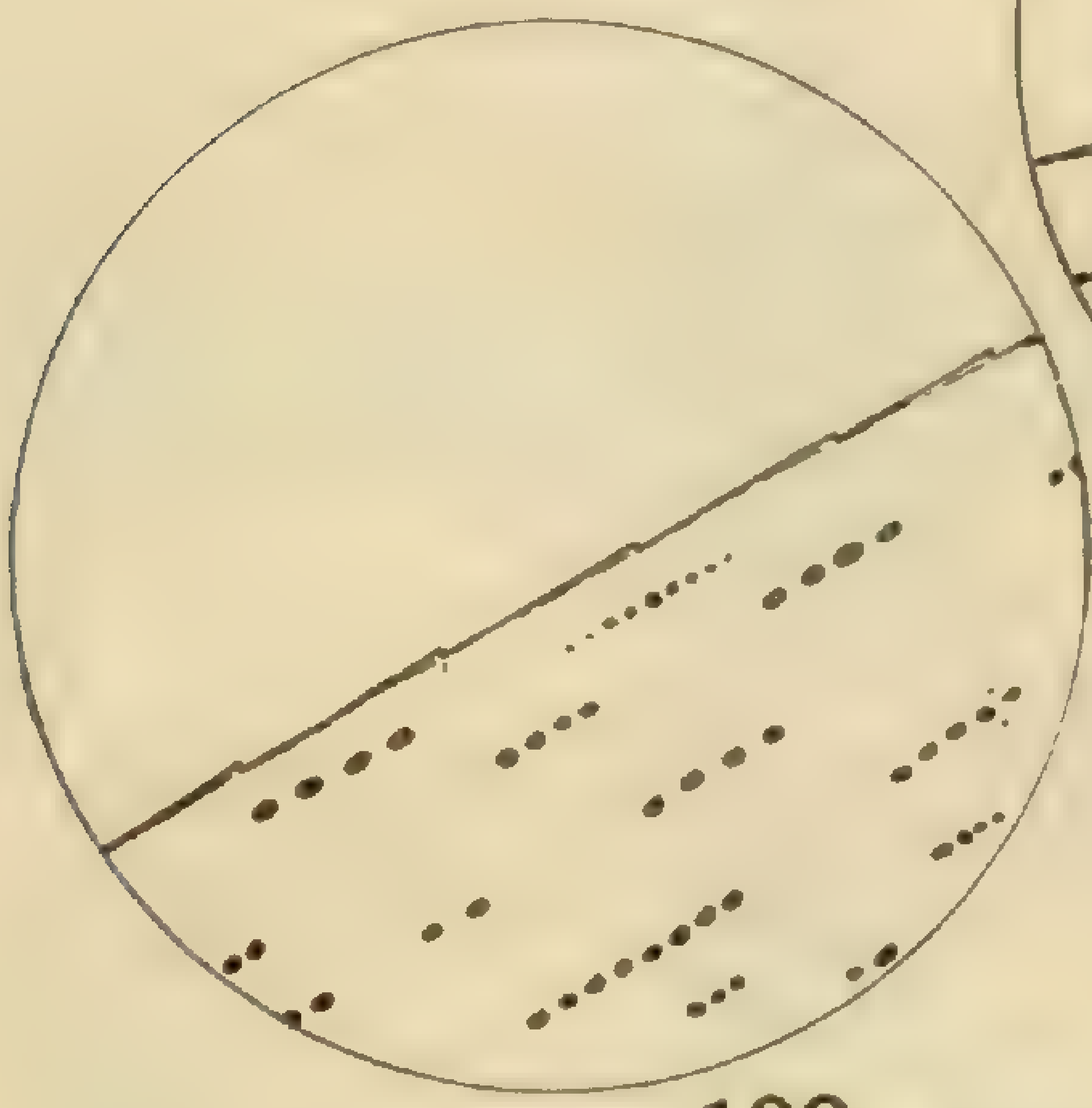

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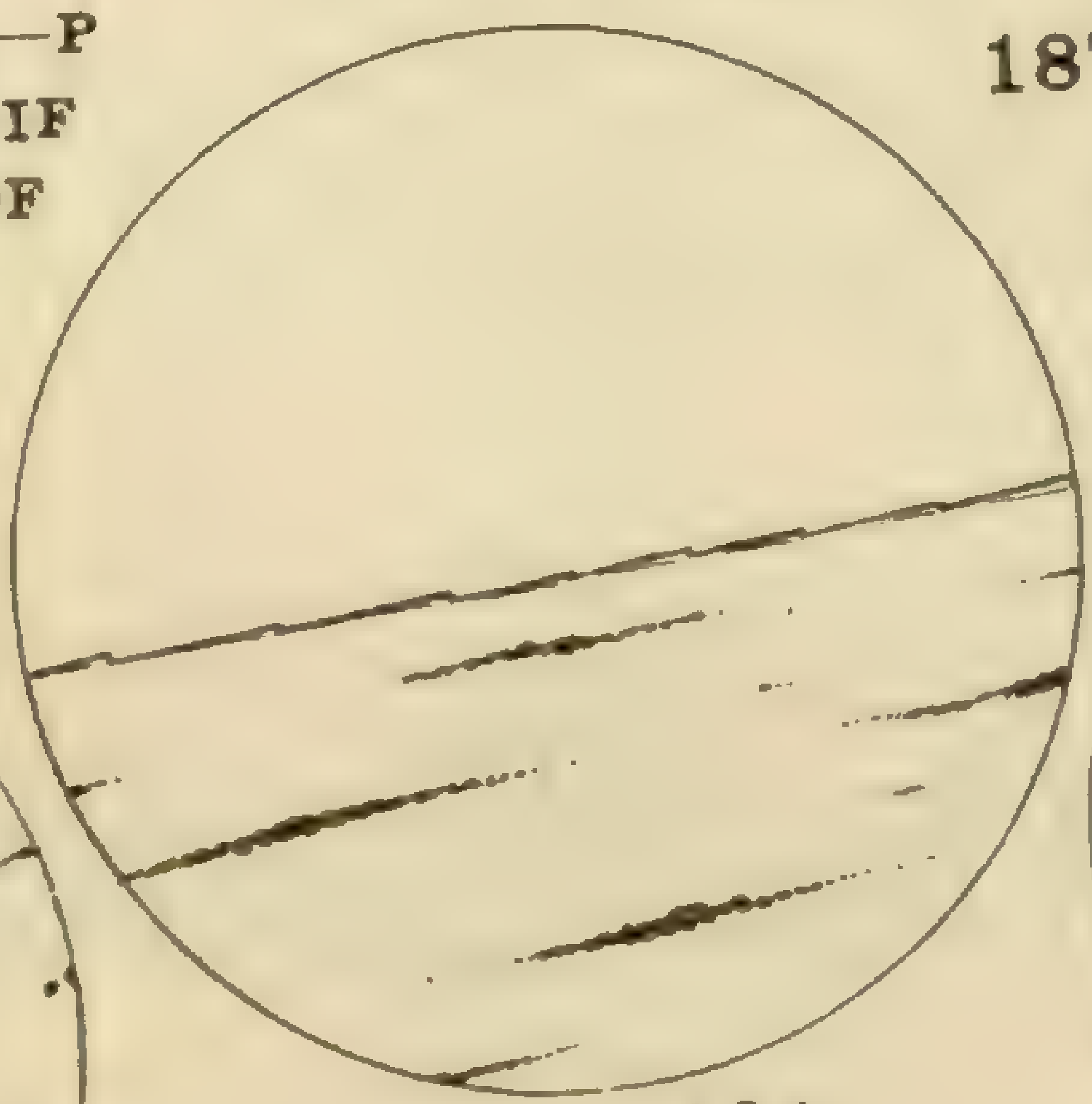

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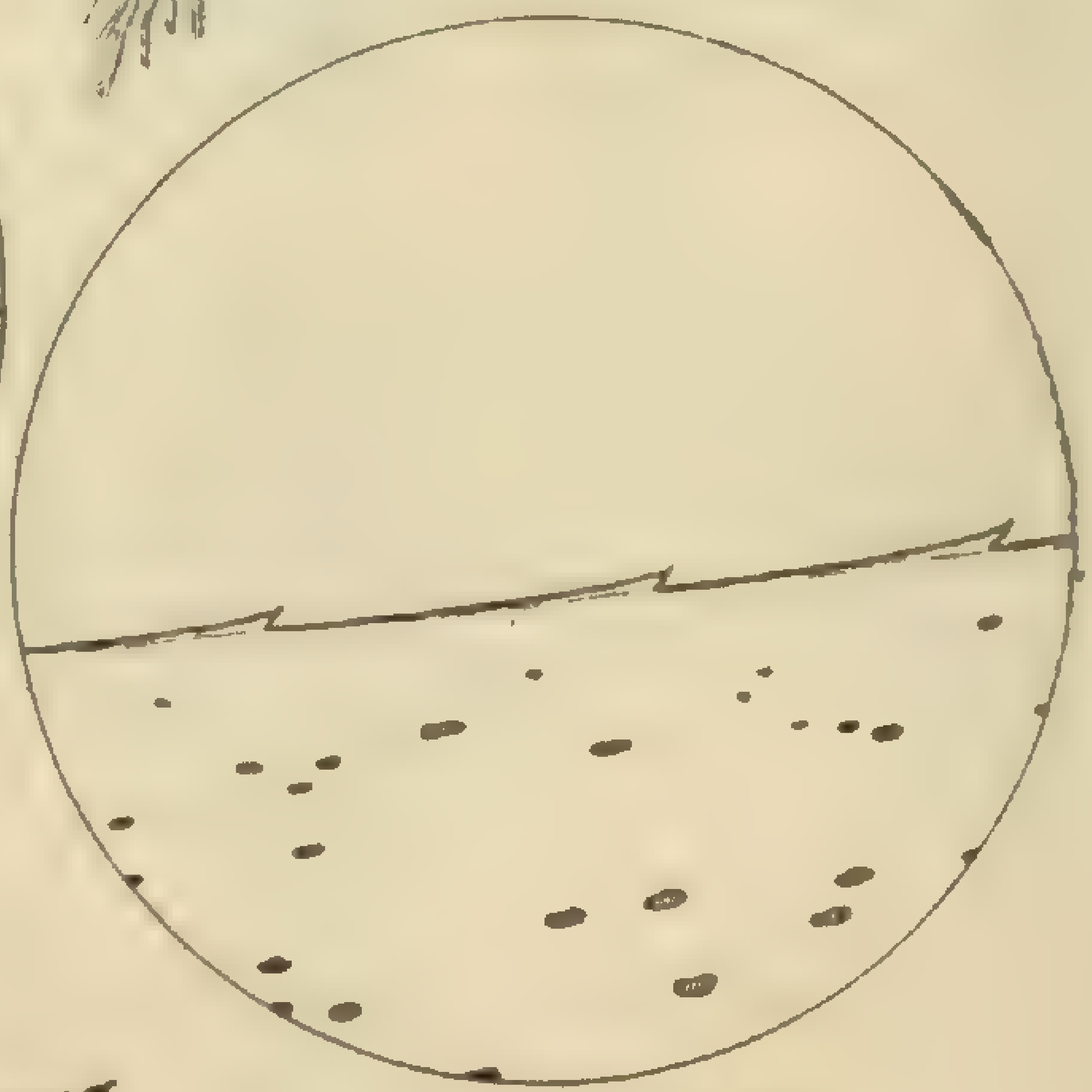

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EXPLANATION OF PLATE VII

FIG. 167. Longitudinal section through an ideal generalized mammalian hair, of the discontinuous medulla variety, to show the relation of its structural elements.

*CU*, cuticle,
*CO*, cortex,
*MC*, medullary cell or chamber,
*I*, interstitial medullary space,
*MS*, medullary shaft or column,
*FCU*, free ectal edge of cuticular scale,
*P*, pigment granules.

FIG. 168. Stereogram of ideal generalized mammalian hair of the continuous medulla variety.

*CS*, cuticular scales,
*C*, cuticle,
*CO*, cortex
*M*, medulla,
*P*, pigment granules.

FIG. 169. Protective hair of European Beaver (*Castor fiber*) to show cuticular scales.

FIG. 170. Protective hair of European Beaver (*Castor fiber*) to show medulla.

FIG. 171. Fur hair of European Beaver (*Castor fiber*).

FIG. 172. Fur hair of Platypus (*Ornithorhynchus anatinus*) just above the mouth of the follicle.

FIG. 173. Fur hair of Platypus (*Ornithorhynchus anatinus*) one third of the distance from the base to the tip.

FIG. 174. Fur hair of Platypus (*Ornithorhynchus anatinus*) near the distal extremity, or tip.

FIG. 175. Protective hair of Skunk (*Mephitis mephitica*) to show cuticular scales.

FIG. 176. Protective hair of Skunk (*Mephitis mephitica*) to show medulla.

FIG. 177. Fur hair of Skunk (*Mephitis mephitica*).

FIG. 178. Stereogram of an ideal generalized mammal hair in its follicle.

*C*, cuticle,
*CO*, cortex,
*M*, medulla,
*SC*, stratum corneum of epidermis,
*SM*, stratum malphigii of epidermis,
*D*, dermis, corium,
*G*, sebaceous gland,
*A*, muscles which erect the hair shaft,
*I*, inner layer of root sheath,
*O*, outer layer of root sheath,
*P*, papilla,
*IF*, inner layer of follicle,
*OF*, outer layer of follicle,
*B*, blood and nerve supply to the bulb of the hair.

FIGS. 179 to 186. Various types of imbricate scales (referred to in text).

FIG. 187. Hair shaft showing teased-out cortical element.

*CO*, cortical cells or hair spindles.

FIG. 188. Transverse section through hair with compound medulla.

*CU*, cuticle,
*M*, medulla,
*CO*, cortex.

FIG. 189. Transverse through a hair with simple or single medulla.

*CU*, cuticle,
*M*, medulla,
*CO*, cortex.

FIG. 190. Portion of shaft of fur hair of Mandril (*Cyanocephalus maimon*) to show the configuration and disposition of the pigment granules.

FIG. 191. Portion of the shaft of the protective hair of the Badger (*Taxidea americana*) to show the configuration and disposition of the pigment granules.

FIG. 192. Portion of the shaft for the fur hair of Wolverine (*Gulo luscus*) to show the configuration and disposition of the pigment granules.

FIGS. 193 to 199. Various types of corneal scales (referred to in text).

under examination, since the form of the scale (more especially) undergoes alteration from the base to the tip of the hair shaft. As a rule the scales at the base of the hair are of greater longitudinal than transverse diameter, while the converse is true of the scales at the tip of the shaft. Figs. 172, 173 and 174 illustrate the nature of the change in form which is normally met with in the hairs of mammals as it occurs in the fur hair of the platypus (*Ornithorhynchus anatinus*). This modification in the form of the scales is believed to be due to the increasing amount of wear to which the hair shaft is subjected the farther away it is pushed from the follicle. That external friction is the cause of scale alteration in form is likewise suggested by the fact that the stiffest hairs possess, usually, scales of a much flattened type (cf. Figs. 57 to 67, inc.), while the finer hairs show the delicate, free ectal edges of the scales unchanged for at least the proximal three fourths of the length of the shaft. This is especially well illustrated in the hair of the bats, notably in such species as the mastiff bat (*Molossus sinaloæ*) (Fig. 105); the wrinkled-lipped bat (*Nyctinomus bocagei*) (Fig. 106); and the intermediate bat (*Mormops intermedia*) (Fig. 107).

The fur hairs shown in the plates[6] were chosen with the view of bringing out most clearly the nature of the forms of the simple varieties of scales and medullas, and of their various common modifications, as they exist one third of the distance from the mouth of the follicle to the top of the hair shaft. For convenience, therefore, the scales and medulla in this portion of the hair shaft have been termed *mature scales* and *mature medulla.* The scales at the distal extremity of the hair shaft, whose modification in form is considered to be the result of attrition, are called the *attritional scales,* and the pinched out medulla of the same region, the *fragmentary medulla.*

Inasmuch as the hair shafts represented in the plates

[6] The fur hair shown in the plates were taken, where possible, from the region of the median line of the dorsum, just below, *i.e.*, caudad of, the shoulders.

vary so widely in diameter (6.80 $\mu$ in the hair of the intermediate bat (*Mormops intermedia*) (Fig. 107); and 1,177 $\mu$ in the hair of the dugong (*Dugong dugong*) (Fig. 159), to draw them to the same scale, and at the same time to make the smaller hairs of sufficient size to show clearly the cuticular scales and medullas, was obviously impracticable. The arbitrary expedient was therefore adopted of drawing all the hairs whose diameters were equal to, or less than, 50 $\mu$ to one size, and drawing all those hairs whose diameters were greater than 50 $\mu$ to another size. In the figures the latter hairs are represented as being slightly greater in diameter. Such a division into coarse and fine hairs is not without its basis in common use, for it was found that as hairs are greater or less than 50 $\mu$ in diameter they are called respectively coarse or fine, or stiff and soft, by perhaps the majority of persons. The true spines form still a third division, with which, however, we shall have nothing to do.

Such an arbitrary representation of hair shafts, however, affords no appreciation of the relative or actual magnitudes of the hairs. In order that this might be had, therefore, the actual diameter of the fur hair of each species, in micra, is given after the name in the explanation of the plates. In each case this, obviously, is approximate only, the result of averaging a large number of individual measurements. It was found that the diameters of the hair shafts of any given individual vary considerably, and that a somewhat less range of variation occurs among the averages of different individuals of the same species. Hence it is inferred that only a meager amount of significance should be attached to hair magnitudes, except possibly, in large averages, and between large groups, *i.e.*, families or genera.

It must also be borne in mind that the prepared hair shaft, underneath the microscope, does not reveal at any one time the complete contour of the cuticular scales, or medulla, as it is represented in the figures. This is due to the fact, that with the objectives of sufficiently high

power to resolve the scale outlines, or the structure of the medulla, but one portion of the cylindrical hair shaft can be brought into exact focus at a time. The objective must in focusing *follow around the hair,* as it were, up one side, and down the other, revealing, as it goes, the course of the outline of the scale, or of the irregularities of the medulla. The resulting curves are then drawn on the single plane of the paper, as though the hair had, by some means, been crushed out flat without distorting its structure. It is because of this rotundity of the various elements of the hair shafts that it is often impossible to secure adequate photomicrographs of hair shafts, since it is necessary to employ high-powered objectives with a consequent very limited focal depth. Moreover the various different indices of light refraction and reflection among the hyaline elements of the shaft produce, upon the finished photograph, various striations and markings of one sort and another, which have no analogue in the actual structure of the hair shaft itself. It is possible, however, that photomicrographs of small, highly magnified portions of the hair shaft, cortex may be very useful in determining the form and placentation pattern of the pigment granules.

The figures of the fur hairs are arranged with the simple form of each type of scale, or medulla, coming first, followed by its various common variations. The hair of the civet (*Arctogalidia fusca*) (Fig. 1) represents the simplest form of the imbricate scale, termed the ovate. Fig. 179 shows the normal appearance of a single isolated scale of this type. Figs. 2 to 7 show the commonest modifications which the ovate scale undergoes. Of all of the imbricate scales whose longitudinal axis is equal to, or greater than, the transverse axis, the ovate is the most common.

Between the ovate scale and the acuminate, no definite line of demarcation can be drawn. I have considered Figs. 8 and 9 to represent perhaps the simplest form of the acuminate type. Figs. 10 to 17 show scales of in-

creasing acuminateness, while Figs. 18 to 20 show curious anomalous varieties.

In Figs. 181 and 182 are shown two isolated acuminate scales of characteristic outline.

The elongate type of cuticular scale (Figs. 21 to 35) is one least often met with, especially in its typical form, as shown in Figs. 29 to 31. The simplest variety (Fig. 21) possesses a longitudinal axis only a trifle greater than the transverse one. Figs. 29 to 31 are the typical varieties, and Figs. 32 to 35 show forms difficult to group. They are, however, tentatively put with the elongate forms. A single dissociated elongated scale is shown in Fig. 183.

By far the commonest types of scale which one encounters are the crenate and flattened types. The former are illustrated by Figs. 36 to 67. In this form of scale the transverse axis is much greater than the longitudinal, and the free ectal, or outermost edge of the scale is irregularly waved or crenulated. Of this type, a confusing multiplicity of variations occur. Some of the plainest and most easily interpreted of these are shown. Fig. 36 is considered to represent the simplest form. Scales like those shown in Figs. 57 to 67 are usually associated with the hairs of the greatest diameter, *i.e.*, the coarse, or stiff hairs, or bristles. This form is also characteristic of the majority of the spines. Two typical crenate scales, dissociated from the cortex, are represented by Figs. 185 and 186.

The flattened type is equally common and differs from the crenate only in exhibiting an ectal edge smooth and comparatively free from sudden irregularities. The longitudinal axis, however, is frequently but little greater than the transverse one, as can be seen in such hairs as are represented by Figs. 69 and 70. Fig. 68 represents the simplest form, and Fig. 184 a single scale of the same type.

In the coronal scale we have a scale fundamentally different from the imbricate. Here the scale usually com-

pletely surrounds the hair. The cuticular portion of the hair may be likened to a pile of tall tumblers placed one within the other, the upper rims representing the free ectal edges of the scales. Isolated coronal scales of various types are represented in Figs. 193 to 199. Fig. 93 represents a form which may be regarded as one of the simplest of the coronal scales. An isolated scale of this form is also shown in Fig. 193. The numerous variations of this type of scale are usually in the direction of a more flaring and more irregular ectal edge, as can be seen by comparing Figs. 93 to 113, and Figs. 193 to 199.

The coronal scales may be subdivided into simple (Figs. 93 to 102), serrate (Figs. 103 to 107), and dentate (Figs. 108 to 113). The simple scales, as well as the serrate are the forms usually found among the bats, which are fairly constant in this regard. Figs. 106 and 107 represent, perhaps, the maximum of scale decoration among the mammals. These scales, isolated from the cortex, are shown in Figs. 196 and 197. The intermediate bat (*Mormops intermedia*) whose hair is illustrated by Figs. 107 and 197, possesses, possibly, the finest of mammalian hair. The shafts of the fur hair average 6.80 $\mu$ in diameter, and often shafts of as small a diameter as 4.30 $\mu$ can be found. In these hairs, apparently, the cuticle has become greatly thickened, and the medulla has been lost. This seems to be true of the majority of the bats, more particularly of those bearing the serrate type of cuticular scales. The dentate type of scale is not found among bats, but seems to be scattered among several orders of mammals. It occurs most frequently among the members of the glires, or rodents. The simplest form is shown in Fig. 108, and other typical forms in Figs. 109 to 112. There seems to be not a great range of variation in this type of scale, the majority of species which bear this type of hair approximating very closely to the forms shown in Figs. 109 to 112. Fig. 113, however, shows an anomalous form of scale characteristic of both the American and European species of otter. In

this form the scale reaches its greatest length, as can be seen by the isolated scale, Fig. 198. The shorter scale, of the usual dentate type is shown in Fig. 199.

Of the three great groups of medullas: the discontinuous, the continuous, and the fragmental, the first seems to be subject to the greatest range of modification. This has been subdivided into simple, and compound types. The simple, furthermore, can be grouped as: ovate, represented by Figs. 114 to 126, elongate, shown by Figs. 127 to 128, and flattened, illustrated in Figs. 129 to 135.

The ovate type, in its various modifications, is met with usually, in hairs of small diameter. Thus the hairs of the shrews, moles, small rodents, one or two bats, etc., possess hairs of ovate medullas. The form usually encountered is apt to be more nearly like those shown by Figs. 114 to 118, than like the remainder of the ovate types (Figs. 119 to 126). The latter, especially such partially fused forms as shown in Figs. 120 and 121, are infrequently seen.

Still less common than these forms are the forms of the elongate medullas (Figs. 127 to 128). These must not be confused with the various fragmental types (Figs. 155 to 166). In the latter the divisions do not represent regularly placed cells or chambers as in the former.

The compound medullas, at least in the fur hairs, are the least common of all. Two varieties can be easily distinguished; the cells of one being ovate (Fig. 136), and the cells of the other flattened (Fig. 137). No instances of elongate cells were observed.

The continuous medulla (Figs. 138 to 153) seems to be the one characteristic of more than half of mammal hairs, particularly of those which are greater than 50 $\mu$ in diameter. It is found in nearly all of the protective, or over hairs, and is present in all spines and bristles, in some portion of the shaft. Fig. 168 shows a hair of this type as it would appear if sectioned to show the longitudinal and transverse appearance of the continuous medulla. The whole interior of the medullary column or

shaft (*MS*, Fig. 167) is filled with an anastomosing mass of cornified filaments, which probably represent a closely compressed aggregation of small medullary cells (Fig. 178). A type of medulla in which the component cells are still preserved so that their individual nature can still be seen, is shown in Fig. 147. Two divisions of the continuous medulla can be readily recognized; the nodose (or irregular) (Figs. 138 to 147), and the homogeneous Figs. 148 to 153). Between these two forms, all sorts of intergradational varieties exist.

The fragmental medulla (Figs. 155 to 166) represents perhaps various stages in the reduction of this element of the hair shaft structure, and seems to have been derived from the continuous type. Where the medulla seems to be lacking altogether, minute traces can still be found in various portions of the hair shaft, particularly in the region just below the mouth of the follicle. Structural indications seem to suggest that the development of medullas is from the discontinuous, through the continuous, to the fragmental, and finally, as is the case in the bats, to no medulla at all.

To prepare hairs for microscopical examination care must be exercised that the reagents used in cleaning, staining, etc., do not soften the cuticle, and thus distort the form of the scales, or that the cover glass is not made to press too heavily upon the hair, and thus flatten it out, deforming both the cuticular scales and medulla as well.

The simplest treatment for scale examination consists in washing the hair thoroughly in a solution composed of equal parts of 95 per cent. alcohol and ether (or chloroform). The hair may then be dipped into pure ether, or chloroform, to insure rapid drying, and when thoroughly dry placed upon a slide and covered with a cover glass for immediate examination. Some hairs, *e.g.*, those of sheep of most varieties, the fur hair of the camels, and the protective hair of many of the bats, notably the silvery bat (*Lasionycteris noctivagans*), exhibit the scales very well after this simple treatment. The 8x or 10x eye-

piece, and the 4 mm. objective with transmitted light, preferably from a blued glass, or better, daylight glass, gives the best results. Indirect lighting, with the mirror swung to one side, may be used where the scale edges are not easily seen. Reflected light has been found excellent, but only in a very few cases.

With hairs like those of the rabbits and hares, shrews, moles, the fur hair of bats, and the like other manipulations of the hair must be brought into requisition. One of the most generally useful of the various staining preparations consists in immersing the hair, after its ether-alcohol bath, in a solution of gentian violet, methyl blue, methyl green, or safranin, in 95 per cent. alcohol. The stain is prepared by making up a saturated solution of the stains enumerated, and then diluting each with 95 per cent. alcohol to the desired degree of color depth, which must be empirically determined for each different species[7] of hair. The evaporation of the alcohol, which must be accomplished rapidly in a warm current of air from a bunsen flame, deposits in the depressions just ectad of each cuticular scale edge, a tiny bit of the stain, which therefore clearly outlines the contour of each individual scale. This method is difficult, and the writer has found that repeated trials with the same hairs were frequently necessary before satisfactory results were secured. In working with hairs it is better to use a tuft of 25 or 50, rather than try to work with but a few.

The preparation of the hair, is, however, of but slight importance compared with the manipulation of the proper lighting and the proper combination of objective and ocular. Where the cuticular scales remain obstinately invisible, or only faintly seen, various sorts of illumination must be tried; transmitted vertical light, transmitted oblique light, dark field illumination, reflected light, and polarized light. Dark field illumination,

[7] The writer is aware that "species of hair" is hardly admissible, yet the convenience must be the excuse for its use.

with the 1.8 mm. objective and 4x eyepiece was found excellent for a large number of hairs. It must be borne in mind that, in using this combination of immersion objective with the dark field illuminator, an oil connection must also be formed between the upper surface of the condenser and the lower surface of the slide.[8] Exigency of space forbids the descriptions of the various types of lighting which have been found most satisfactory with the various species of hairs. These must be empirically determined by each investigator. The degree of success obtained with the microscope usually depends as much upon the preparation of the instrument and its lighting, as upon the preparation of what is to go under it for examination.

For examination of the medulla all that has been said regarding lighting, etc., applies. However, the various treatments given the hair and used to render visible the cuticular scales, obscure the medulla. The simplest and most generally useful method of rendering the medulla clear, consists in reducing the visibility of the cuticular scales to as near zero as possible by mounting the hair, beneath the cover glass, in some light microscopical oil, such as oil of bergamot, of cedar, or origanum, of amber, of cloves, etc., after having washed it, as before, in the ether-alcohol solution. Such a treatment renders the hair, in effect, a glassy cylinder, within which the medulla can be clearly seen, provided the cortex is not thickly besprinkled with pigment granules, or rendered dark in color by diffuse pigment. Fortunately most of the fur hairs are lightly pigmented. Many of the protective hairs, however, are so heavily colored that the medulla is partially, or almost wholly, obscured.

Some of the finer hairs can be examined with advantage in a mount of clear water, or xylol. The best treat-

[8] For directions for all sorts of microscope manipulations, apparatus, microscopical principles, etc., consult Professor S. H. Gage's comprehensive "The Microscope, and Introduction to Microscopic Methods and to Histology," Ithaca, N. Y., 1917. A new edition of this valuable work is now ready to leave the press.

ment, however, was found to consist in washing the hair in the ether-alcohol, drying, immersing in xylol, and then mounting in very thin Canada balsam. This makes a permanent mount.

Lighting with the dark ground illumination was found to give the best results in the examination of the external configuration of the medulla.

In the case of hairs where the heaviness of the pigmentation obscures the medulla, or in compound-medullated hairs, or in those cases where an accurate knowledge of the form of the cross section of the medullary column is desired, it is necessary to prepare cross sections of the hair shaft, by the usual methods of imbedding in paraffin or celloidin.[9] Figs. 188 and 189 show the manner in which the form of the medulla is shown in transverse sections, as well as its relations to the thickness of the cortex and of the cuticle.

The methods used to make clear the medulla serve well also to reveal the pigment granules. In examination of the shaft for these tiny bodies the 1.8 mm. objective and the 10x eyepiece with the draw tube of the microscope extended its full length was found to be the lowest power which could be satisfactorily employed. Lighting with daylight glass and a 200-watt tungsten-filled bulb was apparently a necessity.

The cortex, because of its nearly homogeneous structure, was not found to exhibit characters which could be used as criteria for identification. Fig. 187 shows a hair macerated in caustic soda, and with the cortex teased out to show the distorted, elongated cortical cells, or hair spindles.

The use of caustics and strong acids for dissociating the cuticular scales is not recommended. The softening of the scales distorts their form and thus renders them useless for delicate determinative purposes.

It very often becomes necessary to distinguish the dis-

[9] For histological methods consult Professor M. F. Guyer's "Animal Micrology," Chicago.

tal from the proximal end of some one hair shaft. This can be done under the microscope, remembering: first that the image is reversed, and second, that the free edges of the cuticular scales lie always at the ectal, or distal portion of the scale, and so indicate the direction of the distal extremity of the hair. A much more simple method is to rub the hair in question between the thumb and finger, when it will always travel in the direction of the bulb, *i.e.*, in the direction of its proximal extremity. This fact that the free ectal edges of the cuticular scales develop in such a way that they are always directed outward from the animal, suggests that they may afford protection against the intrusion between the hairs, and so on to the skin itself, of foreign bodies, parasites, and water. Furthermore any such extraneous elements which may have gained entrance, apparently would tend to be worked outward away from the skin to the outer surface of the hair covering by the motions into which the hair is thrown by the movement of the muscles of the body during locomotion.

In preparing a series of animal hairs to be used as type specimens for determinative comparisons with unknown hairs it is well to have a series of slides prepared to show the medulla (mounted in balsam as previously directed), and another series of slides with the hairs mounted thereupon in dry cells[9] (washed in the ether-alcohol, and stained or not as each requires), to show the cuticular scales. Since this later method of preparing hairs seems to be attained with little success (too much dust gathering upon the hair, the fibers obscuring the sculpturings of the cuticular scales), it is better, perhaps, to keep a tuft of each species of hair in a small phial or double envelope, and make fresh preparations when necessary. Both the balsam-mounted slides and the untreated hair samples should be filed away following the classification scheme for the scales and medulla given in this paper. This facilitates the immediate selection of the particular group of hairs possessing the charac-

teristics of the unknown sample, and makes identification much easier and quicker. For each species of mammal samples of the hair from several regions of the body should be had, as well as samples of both the fur and protective hairs of various regions. From his own experience, however, the writer is well aware that this is an ideal more easily recommended than realized.

# THE EFFECT UPON THE WHITE RAT OF CONTINUED BODILY ROTATION

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Everyone knows that a rapid turning-about upon the heels usually leads to dizziness and that a like state is induced in the revolving chair or the turn-table of the laboratory or in the merry-go-round of street fairs. It is also known, especially among those who have attempted to analyze the complicated experience of dizziness, that an important constituent of this disturbed state of mind and body is a characteristic movement, to-and-fro, of the eyes. To this ocular twitching, which is sometimes called "nystagmus," is due, in large measure, the apparent swimming movement of surrounding objects. The twitching appears soon after rotation begins and it continues, with characteristic modifications, for a short period after the body comes to rest.

The bodily and mental effects of rotation in man and in other animals have for a good many years been made the subject of investigation by physicists, anatomists, physiologists, psychologists and medical men. It is supposed that rotation produces a specific effect upon the neural end-organs of the semicircular canals, and it is definitely known that, in addition, pretty much the entire organism is involved in the general disturbance. Concerning the ocular movements themselves, a good deal has been learned. We know, for example, that the character and the duration of the nystagmus depend upon a large and heterogeneous group of conditions, among which may be named the general state of the organism, the state of attention, the associative connections, the rate, regularity and duration of the rotational movements, repetition and practise, and other mental and physical conditions. Of

these conditions, we are here concerned with one only, *i. e.*, with the effect of regular and continued repetition upon the ocular movements in question.

It has been commonly observed that long persistence in whirling movements may reduce in intensity the distressing symptoms of dizziness. This reduction under repetition has suggested that the accompanying ocular movements may also tend, under persistent practise, to disappear. The testimony of whirling dancers and gymnasts, who are frequently undisturbed by the swimming and the giddiness, points in this direction,[1] and further evidence, of an experimental sort,[2] has recently been derived from subjects who were rotated about three minutes daily for two or three weeks. At the end of this period the subjects had lost, either wholly or in part, the "after-nystagmus" which usually persists, as we have seen, when the body has come to rest.

Now these experimental results have been sharply criticized by two otologists, Drs. Fisher and Babcock,[3] who are distressed that the stability of such a "reflex reaction" as nystagmus should be called in question. "Clinical medicine has," as they observe, "for years relied upon the permanency and the constancy of reflex phenomena." As for the results just referred to, they set them down as "pathological." Professing to repeat the experiments, but wholly missing the essential point of the method which they criticize, these men have come, not unnaturally, to a conclusion which is not antagonistic to the dogma of the invariable reflex. Despite the miscarriage of their method, however, they do find a certain amount of reduction in time of nystagmus and this reduction they propose to explain by the voluntary "gaze-fixing" of "a few subjects." Although the abortive

[1] Parsons, R. P., and Segar, L. H., "A Correlation Study of Bárány Chair Tests and Flying Ability of One Hundred Navy Aviators," *J. Amer. Med. Ass.*, 1918, 70, 1064.

[2] *Manual of Medical Research Laboratory*, Washington, D. C., 1918, 186 ff.

[3] Fisher, L., and Babcock, H. L., "The Reliability of the Nystagmus Test," *J. Amer. Med. Ass.*, 1919, 72, 779 ff.

attempt of Fisher and Babcock affords no positive evidence against the demonstrated reduction of nystagmus under repetition,[4] it has suggested an *experimentum crucis* which is designed to show that the reduction is *not* an artefact produced by the "wilful gaze-fixing" of inconvenient subjects who acquired "the art of holding the eye more or less fixed voluntarily" upon a "distant object."

We have chosen the white rat as a subject in our crucial experiment. The rat is admirably adapted to this sort of problem. It is docile and easy to handle. The lack of a fovea and of distant vision and the probable absence of all clear-cut retinal images[5] seem to provide the optimal conditions of non-fixation as suggested by the otologists' contentions. On the other hand, the pupil of the rat's eye is easily observed, as well as those portions of the sclerotic coat which project beyond the surrounding cutaneous and hairy tissues.

The following method of rotation and observation was employed. Upon a pivoted wooden platform, 11 cm. × 20 cm., was set a glass bell-jar 11 cm. in diameter and 12 cm. in height. The rat was so placed under the glass jar that its center of gravity lay over the center of rotation. A small motor, governed by means of a friction-brake, served to provide a very regular and easily controlled means of rotating the platform and the jar. Records of the time of after-nystagmus were at first made with a stop-watch, but later with a key connected to an electric signal-marker which registered on a revolving smoked

[4] As a matter of fact, these authors unwittingly furnish the most delicate and unimpeachable evidence *for* the very reduction which they deny. Although they apparently omitted to repeat at each sitting, giving each of the ten subjects included in their Table II only one turning to the right and one to the left, *in every single case the average nystagmus-time is less for the second five days than for the first five days.* That is to say that a *single turning* each day (not a series) is sufficient to reduce the time for subsequent days. The tendency to reduction must, then, be much greater than the first experimenters had contended or supposed.

[5] Vincent, S. B., "The Mammalian Eye," *J. of Animal Behavior*, 1912, 2, 249–255. See table and also references to the literature.

drum.[6] It was found, by preliminary trials, that the appearance of the nystagmus was directly proportional to the *number* of rotations and to the *speed* of rotation. For experimental purposes, an arbitrary choice was made of a speed of ten revolutions in fifteen seconds. Ten trials of ten rotations each were repeated two or three times a day, save for subjects "I" and "J," which were given twenty trials twice a day. The subjects were ten white rats, five males and five females, all about three months old. The functional integrity of the mechanisms of equilibrium[7] was roughly determined by observing the rats' behavior under daily conditions of life, and by throwing them into the air and dropping them. All of the subjects responded quickly and positively to such tests. In the subsequent experiments each rat was rotated a like number of times to the right and to the left, and averages of the duration (in seconds) of the nystagmus after stopping were computed. A comparison of these averages from day to day may be made from Table I.

The outstanding feature of the investigation is the rapid decrease of after-nystagmus from day to day, as is clearly indicated in Table I. Within ten to eighteen periods of rotation the nystagmus had completely disappeared. The number of ocular movements after stopping the platform was also observed. Upon the first rotation for each rat, the number of movements varied between 18 and 25. This number rapidly decreased during the first four or five periods to between 5 and 8, and soon became reduced to a single movement which generally hung on for some time. As the rotation was stopped, the eye gradually moved in the direction of the preceding rotation and then jerked back to normal position. The

[6] This latter method was used by the Psychological Department of the Mineola Research Laboratory. It is a decided improvement over the clinical method of observation by the stop watch. See "Manual," p. 190.

[7] The semicircular canals of Rodentia are well developed and quite regular in form. See Gray, A. A., "The Labyrinth of Animals," 1907, Vol. I, pp. 165 ff.

TABLE I

| Subject...... | A | | B | | C | | D | | E | | F | | G | | H | | I | | J | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Direction of Rotation | L | R | L | R | L | R | L | R | L | R | L | R | L | R | L | R | L | R | L | R |
| Number of series 1 | 6.70 | 7.04 | 5.88 | 6.06 | 5.00 | 5.80 | 5.80 | 6.40 | 5.34 | 5.06 | 6.02 | 4.82 | 4.40 | 4.60 | 5.80 | 4.60 | 5.66 | 5.86 | 5.12 | 6.16 |
| 2 | 5.62 | 5.80 | 4.52 | 4.90 | 4.96 | 4.60 | 5.32 | 5.20 | 4.76 | 4.02 | 4.20 | 5.12 | 4.60 | 4.60 | 6.00 | 5.00 | 4.35 | 4.45 | 3.96 | 4.06 |
| 3 | 4.32 | 4.32 | 4.02 | 4.18 | 4.08 | 4.44 | 4.60 | 4.72 | 4.22 | 3.20 | 3.56 | 3.66 | 3.80 | 3.64 | 4.46 | 5.30 | 4.10 | 3.95 | 3.82 | 3.58 |
| 4 | 3.78 | 4.10 | 3.70 | 3.76 | 3.26 | 3.92 | 4.08 | 4.20 | 2.58 | 1.80 | 3.56 | 2.64 | 4.78 | 4.30 | 3.88 | 4.04 | 3.00 | 2.98 | 3.72 | 3.30 |
| 5 | 3.22 | 3.48 | 3.48 | 3.42 | 2.96 | 2.30 | 2.96 | 3.00 | 2.88 | 2.56 | 3.06 | 2.42 | 5.46 | 5.20 | 3.92 | 4.54 | 2.10 | 2.20 | 3.52 | 3.96 |
| 6 | 2.68 | 2.62 | 2.70 | 2.58 | 1.28 | 1.28 | 1.78 | 1.96 | 1.50 | 1.36 | 1.28 | 1.50 | 4.46 | 4.34 | 4.20 | 4.64 | 1.18 | 1.56 | 2.74 | 2.94 |
| 7 | 2.06 | 2.18 | 2.34 | 2.26 | 1.00 | 1.00 | 1.08 | 1.06 | 1.32 | 1.08 | 1.02 | 1.04 | 4.46 | 4.76 | 3.68 | 5.16 | 0.96 | 1.18 | 2.86 | 3.42 |
| 8 | 1.62 | 1.74 | 1.74 | 1.80 | 0.60 | 0.60 | 1.00 | 1.00 | 0.60 | 0.86 | 1.00 | 1.00 | 4.18 | 3.68 | 3.58 | 3.18 | 0.40 | 0.50 | 2.96 | 2.92 |
| 9 | 1.32 | 1.48 | 1.54 | 1.56 | 0.20 | 0.20 | 0.40 | 0.40 | 0.20 | 0.20 | 0.40 | 0.40 | 4.18 | 3.96 | 4.58 | 4.14 | 0.50 | 0,20 | 2.60 | 2.70 |
| 10 | 1.04 | 1.56 | 1.26 | 1.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.20 | 3.54 | 3.34 | 3.82 | 3.86 | 0.00 | 0.00 | 2.14 | 2.56 |
| 11 | 1.00 | 1.00 | 1.22 | 1.54 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3.72 | 3.58 | 2.96 | 3.42 | 0.00 | 0.00 | 1.60 | 1.58 |
| 12 | 0.80 | 0.60 | 0.60 | 0.60 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3.66 | 3.76 | 3.10 | 3.40 | 0.00 | 0.00 | 1.76 | 1.38 |
| 13 | 0.40 | 0.40 | 0.00 | 0.00 | | | | | | | | | 3.80 | 3.56 | 3.76 | 3.64 | | | 0.20 | 0.80 |
| 14 | 0.00 | 0.00 | 0.00 | 0.00 | | | | | | | | | 3.50 | 3.74 | 3.68 | 3.60 | | | 0.10 | 0.20 |
| 15 | 0.00 | 0.00 | 0.00 | 0.00 | | | | | | | | | 3.32 | 3.08 | 2.54 | 3.14 | | | 0.00 | 0.00 |
| 16 | | | | | | | | | | | | | 2.24 | 1.84 | 2.16 | 2.18 | | | 0.00 | 0.00 |
| 17 | | | | | | | | | | | | | 1.34 | 1.44 | 1.40 | 1.70 | | | | |
| 18 | | | | | | | | | | | | | 0.00 | 0.00 | 0.00 | 0.00 | | | | |
| 19 | | | | | | | | | | | | | 0.00 | 0.00 | 0.00 | 0.00 | | | | |

disappearance of this one movement accounts for the sudden falling off of the time-values at the end of the various series. The average initial time of after-nystagmus for all subjects was 5.57 sec. for rotation to the left and 5.74 sec. for rotation to the right. Other averages testify to this difference in time for the two directions of rotation. This seems to be a genuine case of individual difference quite comparable to similar differences found in human observers.[8]

The fact of decrease from day to day is incontestable. Each column in Table I shows it. It is just as apparent, if, in each day's series, an average of the first two trials is taken and compared with corresponding values for subsequent days. That is, each day begins at just a little lower nystagmus-time than the preceding day began. Furthermore, the decrease is of a characteristic kind. Table I indicates that at least one half of the total decrease commonly occurs in the first few days of experimentation. The exceptions, subjects "G" and "H," will be considered later. In this respect, a "nystagmus curve" is quite comparable with the common "learning curve," save for the absence of plateaus.

Again, the figures make it plain that there is also a general decrease in the time of after-nystagmus within any single day's turning. Table II indicates that this decrease was constant for all subjects, save "G" and "H." An analysis of the material upon which the table is based shows that the main decrease within any single day falls early in the series—a result consonant with the early fall in time from day to day, as just mentioned.

It must be emphasized that any decrease is for one set of conditions only. Only those values are given which resulted when the rat rested quietly on the floor of the rotated platform. Occasionally the rat would stand almost upright, in which case the nystagmus was almost invariably longer. Even after the disappearance of the

[8] See "Manual," *passim*, and also articles in the *J. Amer. Med. Ass.*, cited above.

TABLE II

| Subject | L | | R | |
|---|---|---|---|---|
| | I | II | III | IV |
| A | 2.81 | 2.49 | 2.84 | 2.51 |
| B | 2.84 | 2.30 | 2.81 | 2.51 |
| C | 2.74 | 2.31 | 2.74 | 2.66 |
| D | 3.18 | 2.89 | 3.13 | 3.00 |
| E | 2.59 | 2.48 | 2.50 | 2.02 |
| F | 2.57 | 2.23 | 2.56 | 2.03 |
| G | 3.46 | 3.50 | 3.40 | 3.30 |
| H | 3.26 | 3.41 | 3.44 | 3.55 |
| I | 2.98 | 2.18 | 2.93 | 2.23 |
| J | 2.98 | 2.33 | 3.28 | 2.65 |

Column I, averages of all the first two rotations to the left for all series; column II, the average of all the last two rotations to the left for all series; columns III and IV, the same for rotation to the right.[9]

nystagmus under *usual* conditions, this upright posture induced some after-nystagmus; but it is important to note that the time and the intensity of it were never so great (by more than half) as the original nystagmus in these positions. That is, there seems to be a "transfer" effect from one set of conditions to another. Changing the speed or the number of rotations at any time produced a similar reappearance of nystagmus, but never in its original intensity or temporal duration. Several of the subjects gave a nystagmus varying between twelve and twenty-five seconds when rotated once a second for thirty seconds before the practise series. After the practise series, these values were reduced to about the level of the original values for the rotation-rate used in the investigation, viz., 5–6 seconds. The change of position of the rat during and after rotation had to be carefully regarded; for such a change was frequently responsible for an increase of nystagmus-time that obscured a real decrease.

There are several special points of importance.

1. It has been noted above that subjects "G" and "H" offer certain exceptions to our conclusions. Table I indi-

[9] Mean variations from the averages given in the tables were computed, but since they were not of sufficient magnitude to affect the significance of the figures as given they have been omitted.

cates that the length of their total series was greater than that of any of the other rats. These two subjects were females rotated during the period of gestation. Their rotation was marked by frequent and severe retching movements, defecation, and micturition. The period of gestation of subject "G" was three days short. "G" became too sick during the last reported turning to be used further, and a day later, during which time she did not seem to recover, a litter of two were born. These coincidences point directly to the fact that nystagmus is closely related to the organic condition of the individual rotated and they at least suggest the fruitfulness of further work upon this matter.

2. The fact that the white rat is a nocturnal animal[10] suggested that the time of day might make a difference in values. Accordingly two rats, "I" and "J," were rotated twenty times twice a day, early in the morning and late in the afternoon. The results were as follows:

| | I | J |
|---|---|---|
| Morning trials, rotation to left (ave.) | 2.24 | 2.81 |
| rotation to right (ave.) | 2.37 | 3.17 |
| Evening trials, rotation to left (ave.) | 1.70 | 2.44 |
| rotation to right (ave.) | 1.88 | 2.40 |

The morning nystagmus is invariably longer, the difference being most pronounced early in the series. Additional evidence of this diurnal difference is being sought with both human and animal subjects.

3. Other responses than the nystagmus were scrupulously noted in our observations. During the first days, most of the subjects showed a tendency to excessive defecation and micturition. Frequently the feces were not of the solid character of normal life but were quite liquid, suggesting that the rotation had induced some sort of temporary organic shock. This supposition is supported by the facts that neither micturition nor defecation ever occurred late in the series and that the rats, although

10 Slonaker, J. R., "The Normal Activity of the Albino Rat, etc.," *J. of Animal Behav.*, 1912, 2, 20–42.

hungry, frequently refused to eat immediately after earlier turnings, although later they eat quite readily. Other evidence of a general organic disturbance is found in the violent trembling which frequently seized some of the rats during a given series. This trembling was distinct from that behavior mentioned before which reminds one of nothing so much as the retching of nausea in human beings. I have failed to find a single case of nauseation in the rat which resulted in an esophageal discharge. The retching did not seem to occur so readily if the rat had had food before the rotational period. The trembling was by far the most characteristic performance and was common to most of the subjects. That the trembling was organically based could be determined by holding the rat just after turning. The visceral organs seemed to be convulsed. The eyes were partially closed and the vibrissæ trembled violently because of the trembling of the mouth parts. This behavior occurred for two or three days after the series had been started and in the case of some of the subjects was the last observable response to the rotation.

4. It is difficult to get a quantitative test for these or for more specifically kinesthetic responses. The only test used in this connection was an enumeration of the number of spontaneous movements made before, during, and after rotation, as the series progressed. Prior to all rotation, the exploratory movements are prominent. As rotation takes place for the first time two kinds of response are in evidence. First, the rat may make frantic efforts to move in the direction contrary to rotation so long as the platform moves. When the movement ceases the rat turns and attempts just as vigorously to move in the opposite direction. These attempts always cease in five or six seconds: they seem to reach their term with the after-nystagmus. The other characteristic response is illustrated by those subjects which squat tensely on the floor of the rotating platform with the head turned far in the direction against rotation. There seems to be a specific

inhibition of all movement and a tenseness of position leading to what one might call the "rotational posture." There is no change in the position until the end of the after-nystagmus, save that the head swings to the opposite side as rotation ceases. This second type of response can be easily induced in subjects manifesting the first kind by slightly increasing the speed of rotation. As the series proceeds, the more striking features of the rotational posture begin to drop out. The head tends to swing less and less in the direction opposite to rotation and very early the return movements—even the bringing of the head back to a straight position—disappear. Finally, the original swing itself becomes quite listless and may not occur at all provided the attention of the rat is elsewhere directed. The exploratory movements return slowly, beginning with the post-rotary period and finally entering the rotary period itself.

5. The scratch-reflex affords an excellent indication of the extensity and intensity of the bodily disturbance present during, and subsequent to, rotation. Early in a series, a scratch movement initiated before rotation is suddenly arrested as rotation begins. I have not observed any scratching during the earlier trials of a series. Subject "J" endeavored to scratch on the fourth day's rotation; but the effort was poorly localized and uncoordinated. At the end of the series, an accurately localized scratch-movement was begun and carried to completion by several subjects, and successful attempts were frequently made to cleanse the face, etc., in spite of rotation or stopping. As the exploratory movements came back to their own, the rat frequently stretched up on its hind legs. In such a position, the stopping of rotation caused a definite compensatory reaction on the part of the rat; but there was nothing here to indicate that this was more than a natural response to the effect of inertia. All of the spasmodic and uncoordinated qualities of an early event of this kind were gone. The tendency of some of the subjects to run in the direction opposite to rotation com-

pletely disappeared after a few trials. In brief, the whole series of any one of the subjects here reported displayed an increasing degree of freedom and precision of movement, as time went on. All subjects were tested for equilibration after a series had been completed and all responded to being thrown and dropped just as alertly as before rotation.

It is hazardous to draw general conclusions from an introductory study of this kind. Our problem does, however, bear directly and significantly upon the functional integrity of the equilibratory mechanisms. The facts above presented support the contention that nystagmus is closely related to the other organic responses to rotation and that it is dependent, as are these other responses, upon a large group of factors. Furthermore, we have found that after-nystagmus in the white rat decreases in intensity and duration (*a*) from day to day and (*b*) within the series of a single day. Either intensity or duration may be modified also by certain organic conditions, e. g., nausea, by speed and number of rotations, and by such general conditions as antecedent rest and fatigue. The decrease and disappearance of nystagmus are accompanied by a disappearance of the characteristic rotational posture and of other bodily disturbances, the disappearance being signalized by the reappearance of the usual exploratory movements and by such specific events as the scratch-reflex.

Fisher and Babcock tried to explain away the demonstrated loss of after-nystagmus under repetition (*a*) by charging that the human subjects were "pathological" and (*b*) by referring the observed decrease to a vicious practise acquired by "a few subjects" of "gaze-fixing" upon a "distant object." It is not clear just how their explanation can be extended to the white rats.

# NOTE ON THE "PELVIC WING" IN POULTRY[1]

PROFESSOR WILLIAM A. LIPPINCOTT

KANSAS AGRICULTURAL EXPERIMENT STATION

QUITE recently Beebe (1915) proposed a four-wing theory of the origin of flight in birds in the stages succeeding the arboreal phase of their evolution. Osborn (1918) raises this theory to the position of an alternate with the older two-wing theory developed from studies on the Archæopteryx (see Heilmann, 1913). Beebe bases his theory on observations of nestlings of the white-winged dove (*Melopelia asiatica*) and the domestic pigeon (*Columba livia* Bonn.), an embryo of the jacana (*Jacana jacana*), a living specimen of the great horned owl (*Bubo virginianus*) and studies of photographs of the Berlin specimen of the Archæopteryx.

The purpose of this note is to report the presence of the structure described by Beebe, in certain domestic birds. In reporting his discovery of a "pelvic wing" in nestling birds Beebe (p. 42) makes the following statement:

> Recently while examining the fresh body of a four-days-old white-winged dove in the New York Zoological Park, I observed on its almost naked body a remarkable development of sprouting quills across the upper part of the hind-leg, and extending toward the tail across the patagium just behind the femur. A second glance showed that this was no irregular or abnormally precocious development on the part of the femoral pterylum, but a line of primary like sheaths, many of which had a very definitely placed covert.

He then proceeds to a rather detailed description of the structure which he called a "pelvic wing."

What appears to be the same structure, judging from Beebe's description and from the figures accompanying his paper, may be readily observed on most chicks of the

[1] Contribution No. 14 from the Department of Poultry Husbandry.

American and Mediterranean breeds at three weeks of age or younger and on English and Asiatic chicks a week or two later.

In the routine of describing three-weeks old chicks in connection with certain genetic studies, the writer has noted and recorded the presence of this structure on several hundred individuals. It has seldom been lacking on chicks of the Mediterranean breeds and crosses, and is usually found on chicks of the American breeds, and frequently on those of English and Asiatic breeds of this age. Its non-appearance in chicks of the lighter breeds, by the time they are three weeks old, is usually associated with low vitality and general slowness of feathering. The heavier breeds are naturally slower in passing from the down to the feather stage and its failure to develop in the first three weeks is more frequent.

While not all of the individuals which failed to show the so-called pelvic wing in three weeks were reexamined, many of them were and in every instance the structure was found at some stage of development. The parents of the chicks observed were not only of several distinct breeds and classes but were frequently from widely separated sections of the country. The number of observations made and the various sources of the breeding stock seem to warrant the belief that the "pelvic wing" in the young domestic fowl is of fairly constant occurrence.

It is interesting to note in this connection that the

EXPLANATION OF PLATE I

FIG. 1. Pelvic wing of Rhode Island Red chick hatched February 4, 1919, photographed March 27, 1919. Age fifty-one days.

FIG. 2. Pelvic wing of bronze poult hatched June 7, 1919, photographed July 11, 1919. Age thirty-four days. Some down feathers plucked to show structure more clearly.

FIG. 3. Pelvic wing of Blue Andalusian chick hatched February 25, 1919, photographed March 27, 1919. Age thirty days.

FIG. 4. Pelvic wing of Blue Andalusian chick hatched March 18, 1919, photographed March 31, 1919. Age thirteen days. Down plucked to show structure more clearly. Leg extended.

FIG. 5. The same individual shown in 4, with leg flexed.

FIG. 6. Pelvic wing of White Plymouth Rock chick hatched March 4, 1919, photographed March 27, 1919. Age twenty-three days.

FIG. 7. Pelvic wing of Barred Plymouth Rock chick hatched March 4, 1919, photographed March 27, 1919. Age twenty-three days.

"pelvic wing" is clearly illustrated in two photographic figures of four weeks old White Leghorn chicks in Rice, Nixon and Roger's (1908, pp. 25–26, Figs. 6 and 7) paper on "The Molting of Fowls." The structure is referred to by these writers as "the thigh tract."

In chickens the "pelvic wing" occurs along the postero-ventral border of the femoral or lumbar tract, as described by Nitzsch (1867, Plate VII, Fig. 6) for *Gallus bankiva* and appears to be a part of it. In its development it is synchronous with, or slightly preceded by, the feathers of the humeral tract and has so much in common with the latter as to suggest that the two tracts may be homologous structures of the hind and fore-limbs, respectively. The "pelvic wing" extends across the upper part of the hind limb and a more or less well-marked patagium just behind the femur. The humeral tract extends across the upper part of the fore limb and the patagium behind the humerus.

The appearance of the "pelvic wing" of chicks of different breeds is shown in Plate I, Figs. 1, 3, 4, 5, 6, and 7.

The structure in the Bronze turkey (Fig. 2) is essentially the same as in chickens. This breed is the largest and commonest variety of domestic turkeys and most nearly resembles their native wild progenitors.

In the waterfowl the structure has not been found. This was perhaps to be expected from the figures of Nitzsch (1867, Plate X, Figs. 5, 6, and 7). The birds observed were domesticated Mallard ducks and White Embden geese. In neither could any feathers be discovered which were set off from the others of the femoral tract, either in size, or precocity of development. There is however in both species a group of feathers whose development is simultaneous with that of the feathers of the humeral tract. These are situated on a branch of the inferior tract which extends beyond the breast along the sides of the trunk almost to the knee. The feathers of both these tracts precede the remiges in development.

Nitzsch (1867, p. 146) makes note of the fact that "this

short outer branch (of the inferior tract), and the broad, obtuse axillary tract, constitute the strongest portion of the entire plumage of the trunk.'' The position of these feathers is such as to suggest a pelvic wing to a casual observer. He further (p. 177) calls attention to the unusual development of this branch in *Crypturus,* where it ''passes through the lateral space of the trunk and unites with the extremity of the lumbar tract of the same side.''

The conditions found in *Crypturus* (see Nitzsch's Plate VII, Figs. 11 and 12), ducks, geese and chickens might suggest the possibility that the ''pelvic wing'' in the chicken, and the branch of the inferior tract in the duck and goose are both vestiges of what was once a continuous row of rather large feathers extending from below the shoulder along the edge of the breast and out over the thigh. Such a suggestion, however, presents difficulties if the homology of the pelvic wing and the humeral tract is seriously considered. Judging from Nitzsch's figures of *Crypturus* (Plate VII, Figs. 11 and 12), there is probably no connection between this branch and the humeral tract.

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# SHORTER ARTICLES AND DISCUSSION

## ON THE NUMERICAL EXPRESSION OF THE DEGREE OF INBREEDING AND RELATIONSHIP IN A PEDIGREE[1]

Dr. Raymond Pearl has given in a series of papers ("Studies on Inbreeding," I–VIII) published in the American Naturalist during the years 1913 to 1917 a system of measuring numerically the degree of inbreeding and relationship in a pedigree.

It will not be necessary to describe the method in any length as it may be familiar to most readers or can easily be found in the original papers.

The starting point is the fact, that all inbred individuals, notwithstanding the special system of inbreeding involved, have fewer different ancestors in a certain generation than the greatest possible number.

The degree of inbreeding is measured by the extent of this reduction in numbers of different ancestors. For that purpose a *coefficient of inbreeding* is determined for each generation according to the formula

$$Z_n = \frac{100(p_{n+1} - q_{n+1})}{p_{n+1}},$$

where $p_{n+1}$ indicates the greatest possible number of different ancestors in the $n + 1$st generation and $q_{n+1}$ means the actual number of different ancestors in the same generation.

Plotting the series of values obtained for Z over a base indicating the series of generations, the *inbreeding curve* can be drawn. Maximum values for the coefficient of inbreeding are obtained when continuous brother and sister mating is involved. The brother and sister inbreeding curve has therefore the importance as the limit, which no other inbreeding curve can surpass.

The proportion of the actual inbreeding during a number of generations to the highest possible inbreeding in the same number of generations then offers a fairly good measure for the

[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 12.

total inbreeding. This proportion can be found by determining the proportion of the area included by the actual inbreeding curve in percentage of the area included by the maximum inbreeding curve. The area of the latter can be calculated by integration. The actual curve is usually so irregular that no integration is possible. To get an approximate value the series of values for Z is simply summed. The formula for the *total inbreeding coefficient* is then the following:

$$Z_{Tn} = \frac{100\Sigma \frac{Zn}{Zl}}{F_{Tn}},$$

the $\Sigma$ denoting the summation of all values between and including the limits indicated, and $F_{Tn}$ is the area included by the maximum curve, the same number of generations being involved.[2]

A question of considerable genetic bearing is the degree of relationship between the parents of the individual, whose pedigree is being examined. Relationship is indicated by the reappearance of individuals from the pedigree of one individual in the ancestry of another.

As a measure of degrees of relationship Pearl proposed a *coefficient of relationship*. This is calculated in two slightly different ways according to whether it is measured with the coefficient of inbreeding, where the relationship of the sire and dam of the inbred individual is being calculated, or separately, when relationship of any two individuals is measured. The formulæ are as follows:

$$(1) \qquad K_n = \frac{100(p_{n+1} - q_{n+1}) - ({}_sZ_{n-1} \cdot {}_sp_n + {}_dZ_{n-1} \cdot {}_dp_n}{1/2p_{n+1}},$$

$$(2) \qquad K_n = \frac{100(p_{n+1} - r_{n+1})}{1/2p_{n+1}},$$

where a prefixed s or d indicates that the following letter refers to the sire's or the dam's pedigree only, and $(p_{n+1} - r_{n+1})$ means the number of common ancestors for both pedigrees in the $n + 1$st generation.

As a measure of the proportion of the actual inbreeding due to relationship between the sire and dam of the individual Pearl has proposed to give a series of *partial inbreeding indices* calculated according to the formula

$$KZ_n = \frac{50(K_n)}{Z_n}.$$

[2] The values of $F_{Tn}$ are given in Table I, "Studies on Inbreeding," VIII, 1917 (Pearl).

In this paper I wish to give a modification and some extensions to the method worked out by Pearl with the purpose of bringing all the measurements of inbreeding and relationship on the same scale and using total coefficients based on calculations of areas as the fundamental method in expressing degrees of kinship numerically.

## I. Definition of the Coefficient of Relationship

If we plot the values of the coefficient of relationship obtained in accordance with the above indicated method together with the values of the corresponding coefficients of inbreeding for a given pedigree, we shall find that these corresponding values are not directly comparable as they are not worked out in relation to the same scale. To obtain this we need to change slightly the definition and formula of the coefficient of relationship.

Pearl's definition is the following: The coefficient of relationship indicates the number of ancestors common to both pedigrees of the two individuals whose relationship is being measured in proportion to the greatest possible number of *common* ancestors in this generation.

The proposed definition is this: The coefficient of relationship indicates the number of ancestors common to both pedigrees of the two individuals whose relationship is being measured in proportion to the total maximum number of *different* ancestors in the two pedigress taken together in the generation in question.

The formulæ are now the following:

(1) (When the relationship between the sire and dam of an inbred individual is being measured)

$$K_n = \frac{100(p_{n+1} - q_{n+1}) - ({}_sZ_{n-1} \cdot {}_sp_n + {}_dZ_{n-1} \cdot {}_dp_n)}{p_{n+1}};$$

(2) (If any two different individuals are concerned)

$$K_n = \frac{100(p_{n+1} - r_{n+1})}{p_{n+1}}.$$

The difference between these formulæ and the first mentioned is only that the denominator in the fractions is multiplied by two. The total values, therefore, are exactly one half of Pearl's.

The maximum value of the coefficient of relationship will in every generation be 50, as no more than 50 per cent. of the individuals in a generation of a pedigree can appear in both halves of the pedigree.

## II. The Total Relationship Coefficient

To measure the total degree of relationship during a number of generations I propose to use a total relationship coefficient based on the same common principles as the total inbreeding coefficient.

The areas to be compared are the area included by the relationship curve (corresponding to the inbreeding curve) and the area included by the maximum relationship curve. Now the maximum values of the coefficient of relationship are 50 in every generation beginning at $K_1$ and the area in question is for that reason simply 50 times the number of generations involved.

The formula of the total relationship coefficient for n generations is then the following:

$$K_{Tn} = \frac{100\Sigma_{Kl}^{Kn}}{50n} = \frac{2\Sigma_{Kl}^{Kn}}{n}.$$

## III. Total Relationship Inbreeding Index

To indicate the proportion of the inbreeding that is due to relationship between the sire and dam of the individual, whose pedigree is being examined, I wish to propose a single numerical expression, the total relationship inbreeding index indicating the proportion of the area included by the relationship curve to the area included by the inbreeding curve, the formula being the following:

$$KZ_{Tn} = \frac{100\Sigma\frac{Kn}{Kl}}{\Sigma\frac{Zn}{Zl}}.$$

*Calculation of the Coefficient Indicating the Degree of Inbreeding and Relationship in the Pedigree of the Jersey Bull King Melia Rioter 14th (103901)*

To show the calculation and significance of the described coefficients I have selected the pedigree of King Melia Rioter Fourteenth including eleven generations. Table I gives the

TABLE I

| | |
|---|---|
| $Z_1 = 25.00$ | $K_1 = 25.00$ |
| $Z_2 = 25.00$ | $K_2 = 25.00$ |
| $Z_3 = 37.50$ | $K_3 = 31.25$ |
| $Z_4 = 50.00$ | $K_4 = 37.50$ |
| $Z_5 = 71.88$ | $K_5 = 43.75$ |

| | |
|---|---|
| $Z_6 = 81.25$ | $K_6 = 46.09$ |
| $Z_7 = 90.63$ | $K_7 = 46.48$ |
| $Z_8 = 92.77$ | $K_8 = 46.88$ |
| $Z_9 = 93.65$ | $K_9 = 46.88$ |
| $Z_{10} = 93.85$ | $K_{10} = 46.88$ |
| Total 661.53 | Total 395.71 |

values of the series of the coefficients of inbreeding and relationship for each generation.

The calculations of the total coefficients are now the following:

$$Z_{T_{10}} = \frac{100 \times 661.53}{900.10} = 73.50,$$

$$K_{T_{10}} = \frac{2 \times 395.71}{10} = 79.14,$$

$$KZ_{T_{10}} = \frac{100 \times 395.71}{661.53} = 59.82,$$

Or expressed verbally: In eleven generations King Melia Rioter Fourteenth is 73.50 per cent. inbred, his sire and dam are 79.14

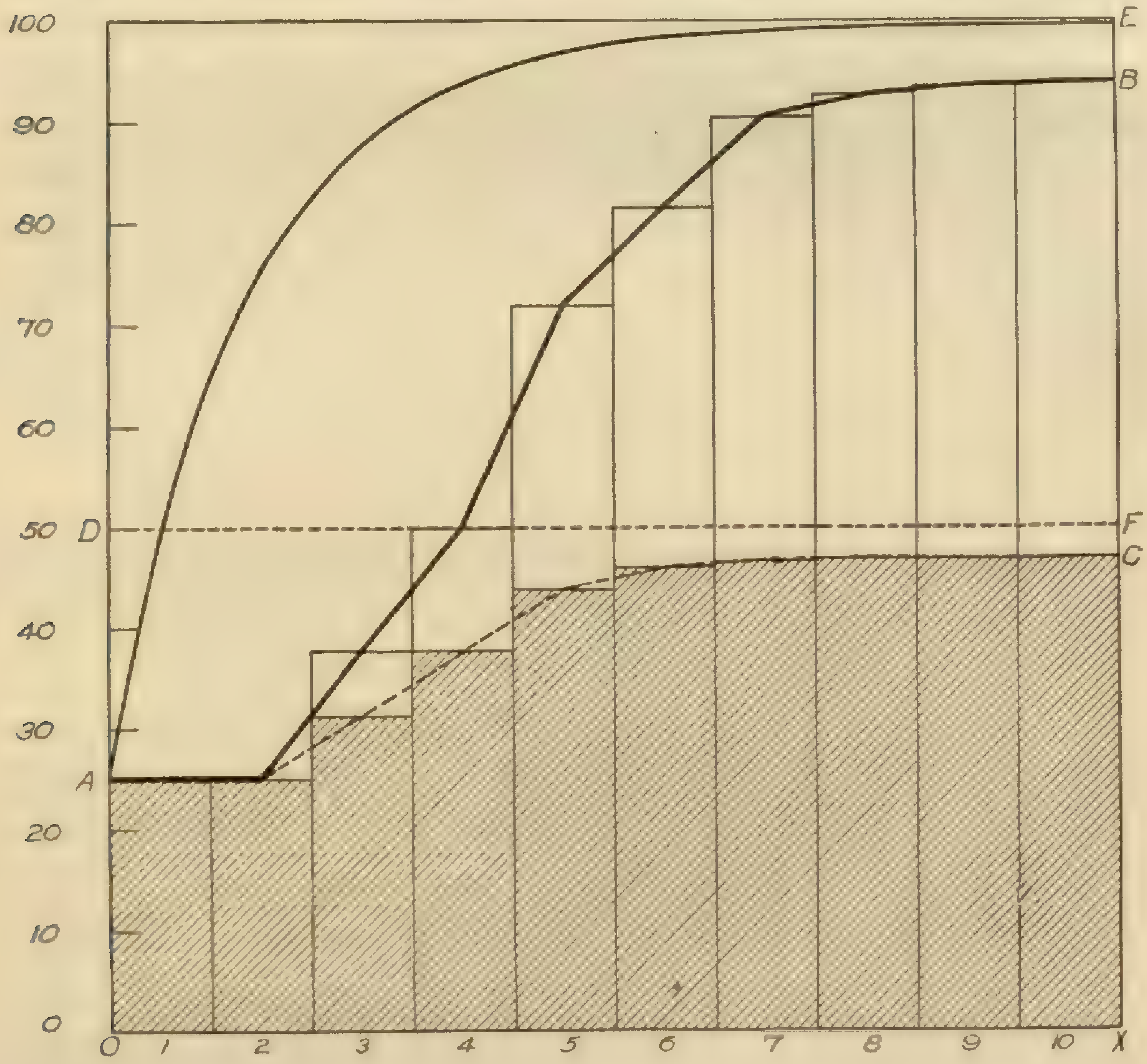


Fig. 1. For explanation see text.

per cent, related, and the part of the inbreeding due to relationship between his sire and dam is 59.82 per cent. of the actual total inbreeding.

In Fig. 1 the inbreeding curve and the relationship curve are plotted, based on the figures given in Table I, the former as a solid line, the latter as a broken line. The smooth curve indicates the maximum inbreeding curve; the broken line, that divides the area in two equal halves, indicates the maximum relationship curve. These four curves taken together give a fairly good graphical demonstration of the facts in question.

1. The area OABX in relation to the area OAEX gives the proportion of the actual to the maximum degree of inbreeding: *The total inbreeding coefficient.*

2. The area OACX in relation to the area ODFX indicates the proportion of the actual to the maximum degree of relationship: *The total relationship coefficient.*

3. The area OACX in relation to the area OABX gives the proportion of the inbreeding that is due to relationship: *The total relationship inbreeding index.*

In bringing all measurements of degrees of inbreeding and relationship to the same scale and using areas as the measures we get a uniform and significant series of coefficients that numerically express the degree of inbreeding and relationship in a given pedigree.

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## SOME OBSERVATIONS CONCERNING THE PERIODICAL CICADA

DURING the recent visitation of the periodical cicada, their great abundance on the writer's home grounds at Vinson Station, Va., afforded an excellent opportunity to observe some of the habits of these interesting insects. During the months of January, February and March, the writer was engaged in clearing off all trees and brush from several lots immediately adjoining his home grounds. In the course of this work, several large oak trees were completely dug up by the roots. Even during the winter months, many of these benumbed creatures were encountered in their burrows in the soil around the roots. As warmer weather approached, their burrows became more numerous in the soil and it was evident that they were approaching the warmer, uppermost layer in ever-increasing numbers. Finally,

on May 18, the first adult was seen making its weak flight over my garden, having emerged some time during the previous night. A few evenings later the great exodus had begun in earnest and thousands of pupæ were issuing from the ground after sundown and ascending all the bushes, trees and posts in the vicinity to transform.

Although the actual exodus from the ground does not take place until after sundown each evening, the pupæ, in preparation for the event, excavate their burrows to the very surface of the ground and await the setting of the sun. In some instances the creatures burrow just to the surface, leaving a very thin layer of soil undisturbed over the exit. Frequently a tiny hole is punctured in the center of this thin surface layer. If these burrows are cautiously approached late in the afternoon long before sundown, the heads of the creatures may be seen near the surface. As the light intensity wanes with the oncoming of evening, the creatures come to the very surface, but quickly retreat if approached or disturbed. It is evident that the pupæ are negatively phototropic. If a pail or box is inverted over their burrows long before sundown so as to exclude the light, the creatures will shortly emerge as if night were really at hand. In this way I have brought many pupæ out of their burrows in broad daylight.

Although the pupæ quickly transform after emerging from the ground, it would be of interest to know just what conditions, external or internal, determine the impulse to prepare for the adult stage. If the creatures are prevented from leaving the ground or soil, the following experiments indicate that the pupæ will remain as such at least a day or two longer than when allowed to ascend trees and shrubs in the normal manner.

On the evening of May 24, five pupæ just emerging from the ground were captured and placed on the damp bare ground beneath a large inverted flowerpot, the drainage hole at the bottom of which had been closed. In addition to these, six other pupæ were captured and placed in a large flowerpot of similar size filled even to the top with loose soil. This pot was covered with a board. Both pots were examined next morning. The pupæ placed on the bare ground beneath the inverted, empty flowerpot were still crawling around, and none had transformed. Of the six placed in the pot containing soil, one had died. The remaining five were alive and active, and likewise none of these had transformed.

On May 25, the following experiment was made with these creatures. Early in the evening before the time had arrived when the creatures usually emerge in response to the low light intensity prevailing after sundown, many were captured by inverting boxes, etc., over the burrows. Later in the evening many more pupæ were captured as they were emerging from the ground in response to the normal darkness following sundown. Six were again placed on the damp earth beneath an empty, inverted flowerpot. Nine were placed as before in a full pot of soil, over which a board was placed to prevent their escape. As controls, six were placed on the branches of a shrub and kept under observation. One of the controls fell off and escaped. The remaining five soon transformed in the normal manner. The next morning, May 26, the pupæ kept beneath the empty, inverted pot and those in the soil were examined. Of the six placed on the bare soil beneath the empty, inverted pot, one had died but the rest were active. None had transformed. All nine pupæ placed in the pot containing soil were alive and crawling over the top of the soil. Likewise none of these had transformed. On the evening of May 26, three of these had died, but the remaining six were as lively as ever. These were then given their freedom and were allowed to crawl up into the branches of a small fringe tree nearby. One fell off and was lost, but the remaining five completed their transformations in the normal manner. Whether this temporary inhibition of the act of transformation is volitional or depends upon some factor of the soil environment acting upon them is not definitely established by these experiments.

After the pupæ ascend the shrubs and trees, rigidity sooner or later takes place, and the adult begins its emergence from the dorsal slit which opens in the pupal skin. It is not long until the lax, soft-bodied creature is hanging head downward by the tip of the abdomen. At this stage of its emergence, when it appears as if the helpless, soft-bodied creature must fall to the ground and perhaps suffer injury, it becomes very active, actually bending up to catch the exuvium or other near object with its legs, just before the tip of the abdomen is released. Not all pupæ are fortunate in their travels and transformations, however, for many come tumbling to the ground from the trees while they are making their way up the trunk and limbs. The almost helpless transformed adults also sometimes fail to secure a foothold and fall to the ground. It is interesting to note how quickly pigmen-

tation is completed after transformation. Immediately after emerging from the pupal shells, the adults are pale yellowish white, with two large conspicuous jet-black areas on the yellowish white prothorax. In a few hours the entire prothorax develops this same black pigment and becomes almost uniformly black.

In some localities the pupæ, in response to special conditions, construct neat little chimneys of earth several inches in height into which their burrows lead. I did not, however, find a single specimen of these unique structures in my locality, although the soil conditions varied greatly.

At Vinson Station, I was afforded an excellent opportunity to observe the occurrence, habits and notes of the dwarf or casinii form as well as the typical, much larger form, since both occurred here. Although the earliest musical expressions of the larger form were heard at my home on May 24, the distinctive notes of the dwarf form were not heard until nearly a week later. A rather well-defined colony of these smaller cicades appeared in some low, shrubby oaks only a few rods from my home, and remained locally abundant here throughout the period of their visitation. It was here that I spent much time in observing their habits. Although the pupæ of the larger and the dwarf form emerged from the ground within the same area in some places, and both forms were singing in the same trees and shrubs, both species appeared to mate among themselves. At no time did I observe a single instance of cross-mating. Although now and then I heard an occasional casinii form singing in the nearby woods, this form confined itself almost entirely to the narrow limits of the shrubby oak growths where it first appeared.

It now remains to consider the "songs" or musical notes of the larger and the smaller forms, for they are entirely different in character. The song of the larger form is a low, and to my ear usually pleasing, droning,—ah-oo—ah-oo—ah-oo—ah-oo—ah-oo. The first, or "ah" syllable is higher in tone and slurs down to the much lower pitch expressed by the syllable "oo." Each phrase "ah-oo" requires about five seconds, and the entire series may be prolonged for many seconds. During the act of "singing," the abdomen is noticeably raised toward the wings on every "ah" syllable, and is lowered on the lower-pitched "oo" syllable.

The "song" of the small Casinii form is a dry, lisping, tone-

less series of sounds, which to me seem best described by the syllables "it-see—it-see—it-see—it-see—it-see—it-see—see—see—see—see." The entire series of notes is hurriedly delivered and does not usually last over 8 to 10 seconds. The first notes of the series—"it-see—it-see"—usually begin slowly and are somewhat subdued in character. The syllables "it-see" gradually increase in loudness, and finally decrease somewhat in intensity, as they run into the shorter, more subduel syllables "see—see—see—see" which terminate the complete "song." The notes of this form are soft and lisping in character, and remind one of the noise of steam escaping intermittently, as one sometimes hears it around a locomotive.

During the height of the "song" season, one could rarely distinguish the notes of any individual, for the myriads of "voices" blended into a volume of soft, murmurous sound—a veritable atmosphere of sound which seemed everywhere to invest the trees and landscape from daybreak till darkness. It was a steady, droning, unceasing hum like the even whirr of machinery. The trees in the National Cemetery were fairly swarming with these creatures and their steady, murmurous chorus could be heard from morning until night, at a distance becoming softened and subdued, and reminding one of the soft murmurs heard when a big sea shell is held to one's ears.

Although the periodical cicada usually becomes silent after sundown, a great nocturnal chorus is sometimes initiated and a remarkable wave of sound invests the night for a time. On the night of May 31, I heard a most memorable, nocturnal chorus of this character, which began just before 2 A.M., solar time. One or two singers in the oak trees in my back yard initiated the concert. Others joined in, and there was a gradual swelling in the volume of sound until it seemed as if all the creatures in these trees were in full song. The concert did not stop here, for I heard it passing on to the big woods toward tulip poplar swamp, until the nighttime was fairly filled with murmurous sound. Gradually the crest of the wave passed outward into the more distant woods, while it subsided slowly in the trees in my back yard where the musical impulse appeared to originate. After some minutes all was quiet again around me, although I could just hear the great wave of sound receding or dying away in the distance. It was the most weird and remarkable chorus I have

ever heard. Hopkins describes a similar instance of this spectacular, nocturnal singing which he once heard.[1]

Many species of birds appeared to find these cicadas especially acceptable morsels. The blue birds in my boxes fed their young upon them extensively, as did a pair of song sparrows which had their nest in a pile of roots in my back yard. House wrens, English sparrows, red-headed woodpeckers and cuckoos fed upon them greedily. Some birds appeared frequently to snap them up in mere play as I once saw a cuckoo doing in the branches of a maple tree over my head. This bird snapped up first one then another in quick succession, quickly dropping them one by one, in a badly injured, helpless condition.

It is of interest to note that individuals differ in eye color. I have noted the following:

1. Males and females with red eye color.
2. Males and females with orange eye color.
3. Males and females with light buff eye color.
4. One male with noticeably white eye color. This individual was distinctive in other respects, since the large veins of the wings, markedly reddish in the common, red-eyed form, were pale yellowish in color. Red-eyed individuals predominate.

Some of the more important dates in the occurrences of the periodical cicada at Vinson Station I have recorded in my journal as follows:

May 18—First adult seen on the wing. The great exodus from the soil began during the next few days.

May 24—First "singing" of larger form heard. First singing of smaller Casinii form heard some days later, about May 27 to May 30.

May 30—Large form in copulation generally. First female noticed laying eggs in twigs.

June 5—Egg-laying activities at their height.

June 14—Creatures becoming very rare, and individual singers only occasionally heard.

June 20—All silent.

June 27—A single, belated individual of the larger form heard in "song."

Although the incessant concerts of the periodical cicadas persisting from morning until night became almost disquieting at

[1] See "The Periodical Cicada," by C. L. Marlatt, Bull. No. 14, Div. of Ent., U. S. Dept. of Agr., 1898, page 58.

times, I felt a positive sadness when I realized that the great visitation was over, and there was silence in the world again, and all were dead that had so recently lived and filled the world with noise and movement. It was almost a painful silence, and I could not but feel that I had lived to witness one of the great events of existence, comparable to the occurrence of a notable eclipse or the visitation of a great comet. Then again the event marked a definite period in my life, and I could not but wonder how changed would be my surroundings, my experiences, my attitude toward life, should I live to see them occur again seventeen years later.

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## THE BEHAVIOR OF FUNDULUS HETEROCLITUS ON THE SALT MARSHES OF NEW JERSEY

During the year 1914–'15 the writer was retained as consulting zoologist to the department of entomology of the New Jersey Agricultural Experiment Station and engaged in studying the fish enemies of the salt marsh mosquitoes. At that time it became evident that *Fundulus heteroclitus* is the most important predatory fish attacking the salt marsh mosquitoes of northern waters. Much evidence of the efficiency of *Fundulus heteroclitus* as a mosquito exterminator has already been published (Chidester, 1916). Certain notes on its behavior under varied conditions have been amplified by more recent observations and are herewith presented in connection with the problem of migration in fishes.

In New Jersey the fish were studied under natural conditions for over a year on the salt marshes near the city of New Brunswick. Through the report system of the state inspectors of the Mosquito Commission, much important information was secured regarding conditions in other parts of the state. Experimental conditions were induced in the field by drainage ditches and in the laboratory by the use of aquaria. Other studies were made at Woods Hole, Mass., for several years during a portion of the month of June.

### Material and Methods

On the salt marshes where the chief study was made there were numerous pools, some permanent, others easily differenti-

ated as temporary. The bottoms of the permanent pools were covered with soft mud and strewn with sedge and eel grass frequently dispersed in windrows as a result of repeated wave action. The bottoms of the temporary pools were covered with matted grass bound together by hardened clay.

At the Bonhamtown marshes near New Brunswick, intensive study was made of three large permanent pools, one of which was partially drained by a ditch connecting it with the Raritan River. Additional studies were made of conditions in many other permanent pools, temporary pools and ditches.

The three permanent pools studied most intensively were quite different in their character. The largest one was about 40 feet long and ranged from a foot to 10 feet in width, but its depth varied considerably with the tides. At its larger end it was connected with the Raritan River by means of a long narrow drainage ditch. The second was an almost circular pool about 25 feet in diameter and in no place more than 18 inches deep; much of the time it was only about 6 inches deep. The third pool was about 30 feet long and about 10 feet wide. At one end it was 20 inches in depth and at the other about 12 inches.

Collections were made by means of a 20-foot minnow seine and several small dip nets. Fish were frequently preserved in weak formalin in the field when it was desired to examine their stomachs at leisure. Usually, however, they were brought to the laboratory and examined freshly killed or else liberated in aquaria.

Records of temperature, salinity and specific gravity were taken with each collection, while the height of the tidal flow and the depth of the pools were approximately recorded.

## Spring Migration

In the early spring, usually during the latter part of March, *Fundulus heteroclitus* begins its migration from the mouth of the Raritan River up beyond the salt water to the slightly brackish water of the salt marshes and even into fresh water creeks.

At first large numbers of medium-sized males appear, followed soon after by the medium-sized females (three or four years old) and later by the large and small of both sexes. By the middle of April great shoals of small fish crowd the streams and penetrate to the shallows. They seem undeterred by the sewage pollu-

tion of the river and are apparently impelled to seek out the farthest limits of tidal water.

Spawning takes place in April and continues until July in the region studied.

The factors influencing inland migration in the spring are several. The *temperature* of the inland waters which is at that time slightly higher than that of the ocean, and will later continue to increase, undoubtedly plays an important part. The fresh water teeming with life and the salt marshes with myriads of insect larvæ, shrimps, and young fish furnish *food* for the vigorous hungry fishes. The currents of fresh water have become stronger and as the fish needs must react to a stream of water not absolutely toxic to it, there is thus a *pressure stimulus* which powerfully attracts. Of perhaps less importance is the fact that the fresher waters when not too greatly contaminated by sewage pollution are *richer in oxygen.* Certain it is that many fish not anadromous come near the shore to spawn. Possibly the greater metabolism incident to the development of eggs and sperm causes them to seek out water which has a higher oxygen content. Roule (1914) believes that salmon migrate to a richer supply of oxygen. Wells (1915) has shown that starvation may cause certain fishes to seek water of lower concentration of salts and other species to behave in the opposite manner.

## Summer Habits

During the summer until early August there is continual migration inland with the tides, many of the fish returning to the brackish water of rivers and creeks as the tide ebbs from the marshes. Some few individuals of the species *Fundulus heteroclitus* find sanctuary in the marsh pools, and in all probability so habituate themselves that they remain until cold weather. From the three permanent pools not partially drained by ditches, collections made during the year furnished the following species.

| Number of Collections | Fundulus het. | Cyprinodon var. | Apeltes quad. | Anguilla |
|---|---|---|---|---|
| 29 .............. | 1,581 | 105 | 19 | 22 |

Since *Fundulus majalis* did not appear on the Bonhamtown marshes it was not feasible to repeat with that species the observations of Mast (1915), who found that it is not only prone to move with the tides, but that if the outlet to the ocean is plugged,

the fish will convey themselves overland by flopping in the general direction of the ocean. Mast shows that the fish are able to keep their sense of direction in the overland course and concludes that they remember the outlet. He believes that since there are apparently no external factors capable of guiding them, the behavior is dependent on internal factors.

*Fundulus heteroclitus* does not as a rule leap from pools, when left by the gradually receding tides. Two permanent pools were available for the study of the reactions of this species, one of them being connected with the Raritan River by a drainage ditch during the course of the study. This pool had been under continuous observation in an undrained condition and through an error workmen ran a drainage ditch to it, which did not, however, completely remove the water. After two days of the resultant condition the ditch was plugged with heavy sods and observations continued as before. The day after the ditch was plugged it was noted that there were many *F. heteroclitus* scattered all around the margin of the large oval pool. Although it was 25 × 15 ft., there was no marked variation in the distribution of the dead fish, except that there were none at the end farthest from the outlet. The banks were gently sloping and afforded an easy egress in any direction. The second pool with an outlet was long and narrow with high banks and was partially drained at the ebbing of the tide. When the receding water had left certain of the fish near the shallows at the exit, there was the usual attempt of the majority of fish to follow an outflowing current. But few individuals were caught in the mud, the majority returning to the deeper pool.

Since there is one predominant reaction in fishes, that to currents, it is quite probable that with *Fundulus heteroclitus* there is a less marked reaction to ebb tide. In the case of *Fundulus majalis* under natural conditions there must be an extremely rapid reaction to the condition of slack tide. Their disturbance under experimental conditions induced by Mast (1915), who plugged the entrance of the tide as it was coming in, indicates that this species does not normally accommodate itself to still water and that its stay inland is determined only by the tidal rise.

In the case of *Fundulus heteroclitus,* which migrates inland to the extremely shallow water covering the salt marshes at high tide, there is no such immediate response to receding water. The

fish return less quickly and seem to become readily acclimated to the still waters of permanent or even temporary pools to which they are directed as the tides recede.

In August there is a period of over two weeks when actively feeding killifish are almost completely absent from the marshes. That temperature plays a most important part in this behavior is indubitable. Shelford and Powers have shown (1915) that the herring is sensitive to temperature differences as small as 0.2° C. They have demonstrated that alkalinity and acidity are more important than salinity. The herring and salmon experimented with reacted to small fractions of a cubic centimeter per liter of $H_2S$ and became negative to sea water which was slightly more acid than the fresh. It is possible that increased temperature may bring into solution organic substances which alter the alkalinity of the sea water or even render it acid near sources of pollution. Johnstone has shown (1908) that the migration of herring in Europe is closely associated with the salinity and temperature of the sea.

We may safely assume that *Fundulus heteroclitus* has an optimum temperature for its metabolism which will be higher when the animal is weak and poorly nourished, but lower when it is well fed. Thus a gradually increasing temperature while the animal is feeding will finally result in such warmth that normal metabolism is no longer possible and there will be no return to the fresher waters until they become cooler. Another factor of great importance in the inland movements of *Fundulus* is the fact that after spawning, the animals are sluggish and hence in no condition for a battle with the tides. This factor is probably the one that causes almost complete disappearance of the larger and the medium-sized *Fundulus* during August in the area studied.

### Fall Migration

Early in September large numbers of *Fundulus heteroclitus* of small and medium size return to the marshes with the tides, and they continue to run in and out until the water becomes extremely cold. There are fewer individuals remaining in the pools between tides, but many are still found variously dispersed among temporary pools far inland.

Their food is somewhat reduced so far as mosquito larvæ are concerned, but there are many other insects available, besides small eggs and shrimps.

## WINTER HABITS

The habits of *Fundulus heteroclitus* in the ocean in winter are not fully known. The late Vinal Edwards of the U. S. Bureau of Fisheries, Woods Hole, Mass., stated to the writer that it was his observation that they spend a large part of the winter near the mouths of rivers in water which is moving and which is at a salinity slightly lower than that of the sea.

In November, when the temperature of the water on the marshes goes down to 40° F., the migration inland is much reduced. Field observations showed that fish in temporary pools at this time attempted to burrow in the bottoms and being of course unsuccessful on account of the hardness of the clay died during the night as the temperature went down to nearly the freezing point. In the case of permanent pools whose bottoms were covered with soft mud, the fish burrowed down during the night and emerged when the sun came out and warmed the water.

At about the time that ice begins to form over the permanent pools, migration ceases so far as the marshes are concerned. In the pools, fish were found burrowed in the mud at a depth of from 6 to 8 inches in the middle of the winter. The temperature of the mud was from 40° F. to 45° F. and that of the water ranged from 32° F. to above 40° F., even in February, since the shallower pools were warmed considerably by the sun. On bright days when the sun was most effective, a few hardy fish ventured forth from hibernation and swam slowly around under the ice, feeding but little. Associated with them were shrimps, myriapods, eels and another minnow (*Cyprinodon* var.), all of which were burrowed in the mud during most of the winter.

Examinations of the stomach contents of *Fundulus* showed that the food during the winter was largely algal matter in those individuals that became active. By far the majority of the fish remained torpid until early spring, beginning to feed again in March, and reassuming complete activity early in April.

## SUMMARY

1. Field studies of *Fundulus heteroclitus* were made throughout one entire year on the salt marshes of New Jersey.

2. Spring migration begins in March and is probably caused by several factors, including the higher temperature of the inland water; currents due to high tides and rainfall; the need

for food available in fresh water; greater metabolic activity due to gonad development which demands a greater oxygen supply.

3. Summer activities consist in spawning, feeding, lazy movements from the marshes to the brackish water and back again.

4. In the autumn, migration is less constant and the larger fish are less numerous.

5. In the winter, migration ceases entirely as the marsh pools are scumming with ice. Some landlocked individuals burrow into the mud of permanent pools, coming out occasionally as the sun warms the water. Many fish are killed by the cold as they remain in temporary pools with bottoms composed of caked mud and grass offering no shelter.

6. The majority of *Fundulus heteroclitus* return to salt water in the winter, probably remaining near the mouths of rivers until spring.

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